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Cost and Performance Report

(ER-201584)

Providing Additional Support for MNA by Including Quantitative Lines of Evidence for Abiotic Degradation and Co-metabolic Oxidation of Chlorinated Ethylenes

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6. AUTHOR(S)

Todd H. Weidemeier
John T. Wilson
David L. Freedman
Brady Lee

5d. PROJECT NUMBER

5e. TASK NUMBER

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

T.H. Wiedemeier & Associates, Inc.
309 Coronado Dr.
Sedalia, CO

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Environmental Security Technology Certification Program
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14. ABSTRACT

The overarching objectives of this project were to:

(1) Provide a method to readily and inexpensively acquire the magnetic susceptibility data required to evaluate the abiotic degradation of TCE by magnetite in aquifer materials using existing non-metallic groundwater monitoring wells.

(2) Provide a method to readily and inexpensively acquire the data required to evaluate and quantify the rate constant for aerobic biological co-oxidation of TCE.

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Radioactive Carbon 14, Distilled Deionized water, Deoxyribonucleic Acid, Dissolved Oxygen, Enzyme Activity Probe, Filter-Sterilized Groundwater, Gas Chromatograph, Nonaqueous-Phase Liquid, Volatile Organic Compound, Abiotic Degradation, Co-metabolic Oxidation, Chlorinated Ethylenes

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ACRONYMS AND ABBREVIATIONS

AFB	Air Force Base
bgs	Below Ground Surface
^{14}C	Radioactive Carbon 14
^{14}C-NSR	Nonstrippable Residue labelled with Carbon 14
^{14}C-TCE	Trichloroethene labelled with Carbon 14
mCi	milli-curie (a unit of radioactivity)
Cinn	*trans*-Cinnamonitrile
DAPI	4′,6-Diamidino-2-phenylindole dihydrochloride
*c*DCE	*cis*-1,2-Dichloroethene
DDI	Distilled Deionized water
DNA	Deoxyribonucleic Acid
DO	Dissolved Oxygen
DOD	Department of Defense
EAP	Enzyme Activity Probe
EPA	Environmental Protection Agency
ESTCP	Environmental Security Technology Certification Program
FSGW	Filter-Sterilized Groundwater
GC	Gas Chromatograph
h	hour(s)
3HPA	3-Hydroxyphenylacetylene
LSC	Liquid Scintillation Cocktail
MCL	Maximum Contaminant Level
mmoZ	qPCR Primer for Soluble Methane Monooxygenase Enzyme
MNA	Monitored Natural Attenuation
NAPL	Nonaqueous-Phase Liquid
O&M	Operation and Maintenance
OU	Operable Unit
PA	Phenylacetylene
PCE	Tetrachloroethene
pH	potential of Hydrogen
PHE	qPCR Primer for Phenol Monooxygenase (Phenol Hydrolyase) Enzyme
PNNL	Pacific Northwest National Laboratory (DOE)

PVC	Poly(Vinyl Chloride)
qPCR	Quantitative Real-Time Polymerase Chain Reaction
RDEG	a qPCR Primer for Ring-Hydroxylating Toluene Monooxygenease Enzyme
RMO	a qPCR Primer for Ring-Hydroxylating Toluene Monooxygenease Enzyme
RPD	Relative Percent Difference
RPM	Remedial Project Manager
SMMO	PCR Primer for Soluble Methane Monooxygenase Enzyme
TEAD	Tooele Army Depot, Utah
TCAAP	Twin Cities Army Ammunition Plant
TCE	Trichloroethene
TOD	qPCR Primer for Toluene Dioxygenase Enzyme
TOL	qPCR Primer for Toluene/Xylene Side Chain Monooxygenase Enzyme
US EPA	United States Environmental Protection Agency
VC	Vinyl Chloride
VOC	Volatile Organic Compound
yr.	year

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The Authors would like to thank the following people. Above all, Environmental Security Technology Certification Program (ESTCP) for funding this project. Second, the Authors would like to thank Dora Taggart with Microbial Insights for donating *q*PCR analyses for this project. The Microbial Insights Quantitative Real-Time Polymerase Chain Reaction *(q*PCR) data allowed quantification of markers that Pacific Northwest National Laboratory (PNNL) could not provide and also provided a check on the accuracy of the data between PNNL and Microbial Insights. – JOHN, PLEASE CHECK THIS STATEMENT. Thanks.

The following people supported T.H. Wiedemeier & Associates, Inc. (THWA) and Scissortail Environmental Solutions, LLC (SES) to coordinate access to the sites and provide field assistance for the sites. Mr. Mark Ferrey with the Minnesota Environmental Pollution Control Agency, coordinated work at the TCAAP in MN. Ms. Diana Cutt with the United States Environmental Protection Agency (US EPA) Region 2 coordinated work at the former Plattsburgh Air Force Base (AFB) and the Hopewell Precision site in NY. Ms. Sharon Stone, AFCEC/CZO, Peterson AFB, Colorado and Mr. Todd Isakson, CH2M Hill, Taylorsville, Utah helped coordinate work at operable unit (OU)-10 at Hill AFB. Mr. Marc Sydow and Mr. Nicholas Montgomery with U.S. Army JMC, provided support for the effort at Tooele Army Depot, Utah. David Farnsworth with U.S. Air Force, Bryan Gamache with AECOM, Lorenzo Thantu US EPA Region 2, Matt Ivers with Parsons, Travis McGuire with GSI Environmental Inc., and W. Zachary Dickson with Dickson & Associates assisted in gaining access to wells and core samples or collecting other data.

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EXECUTIVE SUMMARY

OBJECTIVES

The overarching objectives of the work described herein were to:

(1) Provide a method to readily and inexpensively acquire the magnetic susceptibility data required to evaluate the abiotic degradation of trichloroethene (TCE) by magnetite in aquifer materials using existing non-metallic groundwater monitoring wells.

(2) Provide a method to readily and inexpensively acquire the data required to evaluate and quantify the rate constant for aerobic biological co-oxidation of TCE.

TECHNOLOGY DESCRIPTION

Using mass magnetic susceptibility to predict abiotic degradation of chlorinated alkenes by magnetite in the aquifer matrix has been shown to be viable, but before the work presented in this report, such evaluation required that a core sample of the aquifer material be submitted for laboratory analysis. This report shows that an inexpensive downhole sonde (probe) can be used in existing 2- and 4-inch Poly(Vinyl Chloride) (PVC) groundwater monitoring wells to quantify magnetic susceptibility of aquifer material.

Bacteria that degrade natural organic matter in groundwater contain enzymes (oxygenases) that can aerobically degrade TCE through a process of biological co-oxidation. Bacteria that contain active oxygenase enzymes can be recognized using fluorescent Enzyme Activity Probes (EAPs), and the bacteria can be counted under a microscope. There are primers that can be used in the Quantitative Real-Time Polymerase Chain Reaction (qPCR) to amplify Deoxyribonucleic Acid (DNA) that codes for selected oxygenase enzymes. A qPCR assay can be used to determine the number of gene copies for these enzymes in a sample of groundwater. Aerobic cooxidation is a promising risk management strategy for large dilute plumes, but its application has been limited because the co-oxidation of TCE in the environment is difficult to quantify by simply measuring changes in the concentration of TCE in the field, and the numbers of bacteria in groundwater that have the oxygenase enzymes has not been directly correlated to field-scale rates of degradation.

Because determining field scale rates for co-oxidation of TCE using concentration data is problematic, a Radioactive Carbon 14 (^{14}C) labelled TCE assay was developed to measure rate constants. The utility of EAPs and qPCR assays to evaluate co-oxidation of TCE was determined by comparing the rate constant developed using the ^{14}C-labelled TCE assay to the abundance of cells that react with EAPs or the abundance of gene copies for oxygenase enzymes.

DEMONSTRATION RESULTS

Values for volume magnetic susceptibility were determined in 26 PVC wells using a downhole sonde. The values were converted to mass magnetic susceptibility, and compared to values for mass magnetic susceptibility from laboratory analyses on samples from boreholes that were adjacent to the wells. There was good agreement between the two measurements.

Out of 19 groundwater samples evaluated using the Trichloroethene labelled with Carbon 14 (^{14}C-TCE) assay, TCE co-oxidation could be documented in 8 samples, with first order rate constants ranging from 0.00658 to 2.65 yr^{-1}.

In a particular water sample, the abundance of gene copies of the most common oxygenase was similar to the abundance of cells reacting to the EAPs. Some oxygenase enzymes were more abundant in groundwater from some wells and other enzymes were more abundant in other wells. Cooxidation of TCE could not be attributed to any one oxygenase enzyme. To further complicate interpretation of the abundance of DNA gene copies, not all the DNA in bacteria is actively transcribed to make enzymes at any one time. If the mRNA transcript for an enzyme is present in a sample, that is evidence that the gene is being transcribed.to make the active enzyme. The total abundance of active DNA gene copies was calculated as the sum of the individual gene copies of oxygenase enzymes for which the mRNA transcript was detected. There was a useful relationship between the total abundance of active DNA gene copies and the rate constants for TCE cooxidation. The 80% prediction interval of a regression of the rate constants on the total abundance of active DNA gene copies is only one order of magnitude wide.

COSTS

The cost to determine volume magnetic susceptibility in one well using a down-hole sonde is approximately $2,000. The cost of the ^{14}C assay of the rate constant of cooxidation of TCE is approximately $476 per well. The cost of the EAP assay is approximately $1,900 per well. The cost of the qPCR analyses is approximately $835 per well.

IMPLEMENTATION ISSUES

Laboratory microcosm studies have shown that some aquifer sediments have appreciable values for mass magnetic susceptibility but no evidence for abiotic degradation of TCE. Values of mass magnetic susceptibility should only be used as a second line evidence to support a rate constant for TCE degradation that is extracted from site characterization data, as is illustrated in the decision logic of Lebrón et al. (2015). Mass magnetic susceptibility should not be used as primary line of evidence to extract a rate constant.

Similarly, the abundance of cells that react to an EAP or the abundance of DNA amplified by a qPCR marker for an oxygenase enzyme should be used as a second line evidence to support a rate constant for TCE degradation that is extracted from site characterization data. They should not be used as primary line of evidence to extract a rate constant.

Two other significant implementation issues are the cost of the EAP analyses and the fact that they can only be completed by the Pacific Northwest National Laboratory (PNNL) and the requirement that the ^{14}C-TCE assay be done in a certified and permitted laboratory. A third implementation issue has to do with the integrity of the PVC monitoring wells; specifically, 2-inch groundwater monitoring wells. If these wells are not sufficiently straight, or if the joints are not flush, then the magnetic susceptibility sonde cannot be lowered into the well, and it will not be possible to obtain mass magnetic susceptibility readings in such wells.

1.0 INTRODUCTION

1.1 BACKGROUND

Monitored natural attenuation (MNA) and enhanced bioremediation have gained popularity as remediation approaches at sites contaminated with chlorinated solvents over the past 25 years. Environmental Security Technology Certification Program (ESTCP) Project Number ER-201129 developed a quantitative framework to aid in the selection of MNA or bioremediation approaches (biostimulation alone, or biostimulation combined with bioaugmentation) at sites contaminated with chlorinated ethylenes. Upon completion of ER-201129, two shortcomings regarding the current state of the science were identified, including:

- In some cases, the investigator may not want to expend the resources necessary to fully implement the decision framework developed for ER-201129. The most notable example occurs when the investigator has worked through the decision framework and will not be able to proceed without magnetic susceptibility data. Using mass magnetic susceptibility to predict abiotic degradation of chlorinated alkenes by magnetite in an aquifer matrix has been shown to be effective (ESTCP, 2015; He, 2009). However, before the work presented herein, this evaluation required that a core sample from a borehole be submitted for laboratory analysis. Obtaining core samples at many sites is unrealistic because the drilling program has been completed. Thus, general and widespread acceptance of the approach outlined in ER-201129 was limited. As detailed in this report, this project develops and validates a more affordable technique to measure magnetic susceptibility with a sonde (probe) that can be inserted into an existing monitoring well. The use of a downhole sonde that can be used in existing 2-inch or 4-inch inner-diameter non-metallic monitoring wells should increase the implementability and use of the decision framework developed for ER-201129, including BioPIC.
- Bacteria that degrade natural organic matter in groundwater contain enzymes (oxygenases) that can aerobically degrade trichloroethylene (TCE) through co-oxidation. These bacteria use oxygenase enzymes to degrade organic matter in groundwater. Trichloroethylene is fortuitously degraded by the same oxygenase enzymes that are produced during degradation of native organic matter. This degradation mechanism is promising for large dilute plumes, but its application has been limited because the numbers of bacteria in groundwater that have the oxygenase enzymes have not been directly correlated to degradation rates until now. This degradation pathway was not included in ER-201129 because it had not yet been quantified. This report quantifies the relationship between oxygenase enzymes and degradation rates.

A number of studies have demonstrated that remedial goals can be met with significantly reduced environmental impacts, capital investment, and operation and maintenance (O&M) costs by implementing MNA. The results of this project and the results presented in this report should increase the number of sites where MNA is implemented because it allows the Department of Defense (DOD) to better describe the mechanisms and processes that contribute to natural attenuation. This is because degradation by magnetite and aerobic co-oxidation have largely been neglected when evaluating MNA in the past, which often has resulted in the misinterpretation of degradation mechanisms and thus, the efficacy of MNA.

For example, when abiotic degradation or aerobic co-oxidative degradation are the predominant degradation mechanisms, the investigator may falsely conclude that degradation has "stalled" at dichloroethylene (DCE), typically *cis*-1,2-Dichloroethene (*c*DCE). With the information presented in this report, the DOD and other responsible parties will be able to present State and Federal regulatory agencies with quantitative estimates of the contribution of abiotic degradation by magnetite and aerobic co-oxidation to the overall rate of natural attenuation at DOD sites contaminated with chlorinated ethylenes. An increase in the use of MNA will minimize detrimental environmental impacts, such as greenhouse gas emissions, at sites where unnecessary remediation previously would have taken place. This will reduce both capital and O&M costs to the DOD.

1.2 OBJECTIVES OF THE DEMONSTRATION

The overarching objectives of the work described herein are to:

(1) Provide a method to readily and inexpensively acquire the magnetic susceptibility data required to evaluate the abiotic degradation of chlorinated ethylenes by magnetite in existing non-metallic groundwater monitoring wells.

(2) Provide a method to readily and inexpensively acquire the data required to evaluate and quantify the aerobic co-oxidation of TCE.

Based on the data and information presented in this report, these objectives have been met. It is anticipated that this work will further promote the implementation of MNA where it previously was not implemented because of a lack of understanding of these important degradation processes, and the inability for them to be readily quantified at many sites.

1.3 REGULATORY DRIVERS

Presently, the maximum contaminant levels (MCLs) for the chlorinated ethylenes, e.g., tetrachloroethene (PCE), TCE, *c*DCE, and vinyl chloride (VC) are 5 micrograms per liter (µg/L), 5 µg/L, 70 µg/L, and 2 µg/L, respectively (http://water.epa.gov/drink/contaminants/index.cfm). At many sites, a risk-based assessment dictates cleanup goals, which often means that MCLs are not the regulatory driver. In any event, some type of remedial action is required at many DOD sites where chlorinated ethylenes are present. This project expands on the elucidation of degradation pathways outlined in ESTCP ER-201129 to allow DOD Remedial Project Managers (RPMs) to choose the most efficacious remediation approach to meet remedial objectives.

2.0 TECHNOLOGY

2.1 TECHNOLOGY DESCRIPTION

The results presented in this report allow the efficacy of MNA to be evaluated more efficiently and accurately than it has been in the past using tools that had already been developed but had not been adequately tested for environmental applications. Specifically, the techniques described and quantified in this report benchmark abiotic degradation by magnetite and aerobic co-oxidation at sites contaminated with chlorinated ethylenes. Figure 2.1.1 is a process schematic showing integration of key components of the demonstration.

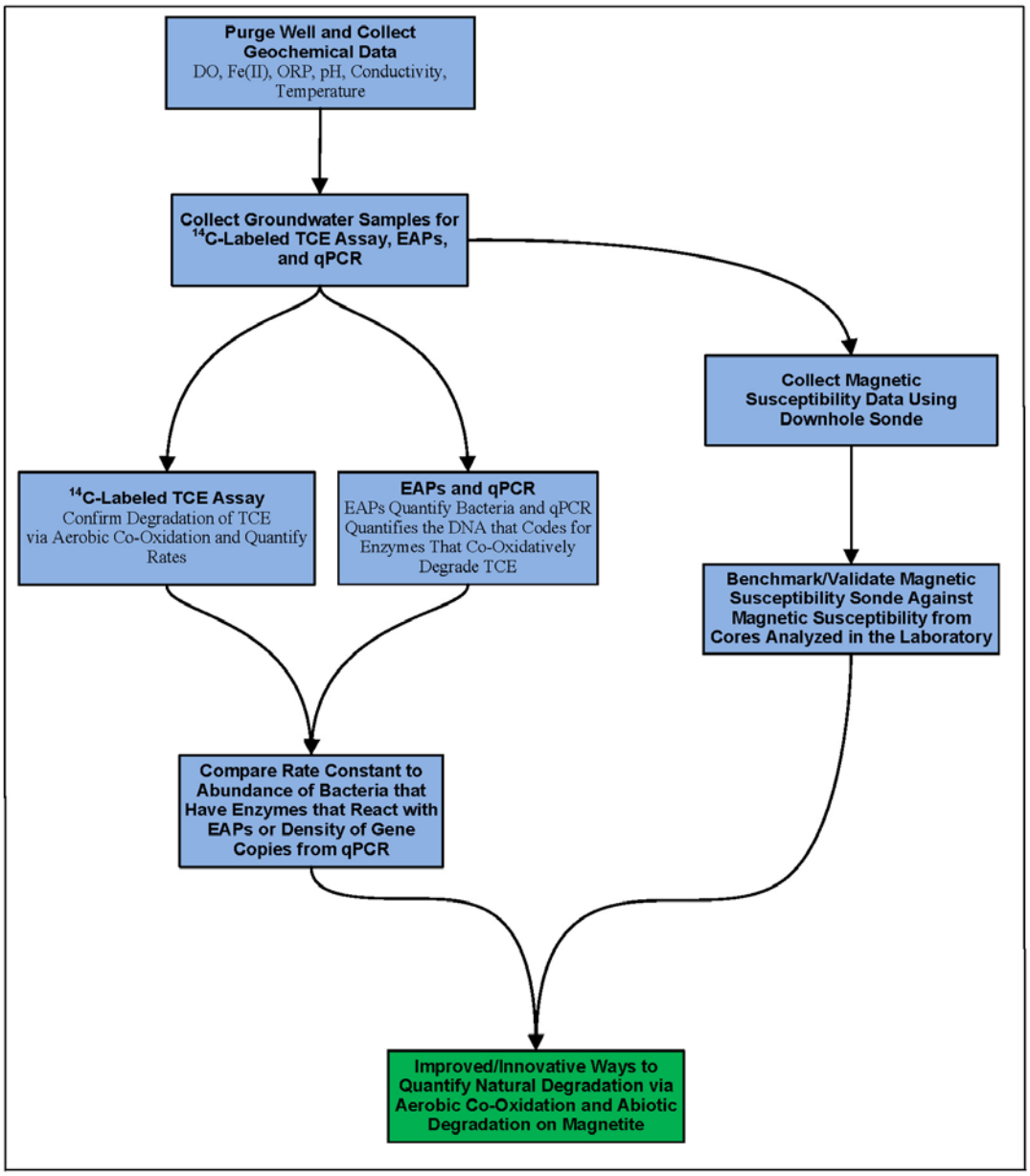

Figure 2.1.1. Process Schematic Showing Integration of Key Technology Components.

Using mass magnetic susceptibility to predict abiotic degradation of chlorinated alkenes by magnetite in the aquifer matrix has been shown to be viable, but before the work presented in this report, such evaluation required that a borehole core sample be submitted for laboratory analysis. This report shows that an inexpensive downhole sonde (probe) can be used in existing 2- and 4-inch Poly(Vinyl Chloride) (PVC) groundwater monitoring wells to quantify mass magnetic susceptibility.

Mass magnetic susceptibility is a useful surrogate for the quantity of magnetite in aquifer material. This is particularly true because it is not possible to directly measure magnetite at concentrations less than 10000 mg kg-1 and most aquifer materials contain less than 10000 mg kg-1 of magnetic materials (He et al., 2009).

Bacteria that degrade natural organic matter in groundwater contain enzymes (oxygenases) that can aerobically degrade TCE through a process of co-oxidation. This degradation mechanism is promising for large dilute plumes, but its application has been limited because the numbers of bacteria in groundwater that have the oxygenase enzymes has not been directly correlated to field-scale rates of degradation, and the co-oxidation of TCE in the environment is difficult to quantify by simply measuring changes in the concentration of TCE in the field. Because determining field scale rates for co-oxidation of TCE using concentration data is problematic, a Radioactive Carbon 14 (^{14}C) labelled TCE assay was developed to quantify degradation rates.

The utility of Enzyme Activity Probes (EAPs) and Quantitative Real-Time Polymerase Chain Reaction *(q*PCR) assays to evaluate co-oxidation of TCE was determined by comparing the rate constant developed using the 14C-labelled TCE assay to the abundance of cells that react with EAPs or the abundance of gene copies for oxygenase enzymes.

2.2 ADVANTAGES AND LIMITATIONS OF THE TECHNOLOGY

Advantages:

The advantages of the methods described in this report include:

- The results presented in this report allow elucidation and quantification of degradation mechanisms that in the past could not be readily quantified using only existing monitoring wells. For example, in the past, soil samples were required to quantify magnetic susceptibility in an aquifer matrix. The use of a downhole magnetic susceptibility sonde circumvents this limitation. Also, as opposed to collecting discrete soil/sediment samples which are discontinuous along the length of the borehole, the use of a downhole sonde allows continuous readings along the length of the entire borehole in which a PVC monitoring well has been installed. This increases the level of detail for magnetic susceptibility measurements across the contaminated aquifer, thus allowing better characterization. The use of a downhole sonde also significantly reduces costs at those sites where exploratory site characterization has largely been completed and there are no plans for a drilling rig to be mobilized to the site in the future.
- Aerobic co-oxidation of TCE by oxygenase enzymes yields products such as CO_2, CO, formate, glycolate, and oxalate. These products are also formed during biodegradation of non-chlorinated and naturally occurring organic matter, and therefore prior to the work presented in this report, it was not possible to distinguish their source.

Furthermore, when the concentration of TCE in the environment is low relative to background organic matter, the concentration of biodegradation products formed from TCE co-oxidation may be very low by comparison. Use of ^{14}C-labeled TCE overcomes this problem, since all of the carbon-based products formed will also be labeled. Even trace levels of products are measurable, because of the extremely strong "signal" from ^{14}C.

- Use of ^{14}C-labeled TCE makes it possible to quantify the rate of TCE transformation with significantly greater precision than simply measuring the disappearance of TCE.
- The assay used for this project only utilized groundwater. It is shown in this report that rates of TCE co-oxidation are quantifiable without having to employ core samples in the assay. The sensitivity of the assay permits determination of transformation rates without the presence of core material.
- Although use of ^{14}C material can only be performed in laboratories permitted to use radioactive material, ^{14}C poses much lower hazards in comparison to other radioisotopes that are commonly used, e.g., for medical applications.
- EAP is a direct measurement of bacteria with active oxygenase enzymes, so few biases are associated with application of the technology. EAP is currently the only technology available to probe activity of oxygenases responsible for co-metabolism of TCE.
- *q*PCR is a proven and commercially available technology for determining the presence of bacteria carrying copies of oxygenase genes responsible for co-metabolism of TCE.

Possible Limitations:

The possible limitations of the work presented herein are:

- The magnetic susceptibility sonde cannot be used in stainless steel wells. Wells larger than 4 inches in diameter may be problematic for collecting accurate magnetic susceptibility data because of the size of the borehole required for such wells. However, larger sondes, with a larger radius of influence, are available.

- As mentioned above, ^{14}C assays can only be performed in laboratories that are permitted to use radioactive material. Furthermore, the cost for ^{14}C-labeled TCE is considerable (~$11,000 per milli-curie [mCi]), mainly because it is no longer available as a stock compound and must therefore be custom synthesized. If the assay is adopted for more frequent use, suppliers may opt to once again provide ^{14}C-labeled TCE as a stock item, which will decrease the cost.

- The ^{14}C assay is not yet commercialized. It is hypothesized that the successful demonstration of the protocol presented in this report will provide considerable motivation for private companies to offer the service. An analogous situation was the use of compound specific isotope analyses (CSIA). At one time, use of this technology for groundwater samples was limited to a select few academic laboratories. As the value of the approach became apparent, commercial laboratories stepped in to meet the growing demand. We anticipate that a similar outcome will develop for the ^{14}C assay proposed in this study.

A detailed protocol to implement the assay is provided in Appendix B. Outside of the ^{14}C assay described in Appendix B, there is no technically viable approach to directly measure a rate constant for the natural biological cooxidation of TCE in groundwater.

- EAP at the current level of development are only a qualitative predictor of aerobic bioremediation, since probe response was never adequately calibrated to the actual rate of contaminant biodegradation in groundwater at field sites. EAP analytical services are currently only available through Pacific Northwest National Laboratory (PNNL).

- qPCR can be affected by biases associated with Deoxyribonucleic Acid (DNA) extraction, as well as issues associated with efficiency of DNA amplification.

3.0 PERFORMANCE OBJECTIVES

Tables 3.1 and 3.2 summarize the performance objectives, success criteria and data requirements for the demonstration. The Final Technical Report provides detailed descriptions of each performance objective.

Table 3.1. Qualitative Performance Objectives

Objective(s)	Data Requirements	Success Criteria	Results
Develop an approach for measuring magnetic susceptibility in existing non-metallic monitoring wells that provides data of useful quality.	Data on magnetic susceptibility from sondes in monitoring wells that can be compared to laboratory measurements of magnetic susceptibility on core samples from the site.	Objective met if users can easily obtain accurate magnetic susceptibility data using a readily-available downhole sonde.	This performance metric was met. Users have access to a commercially-available downhole sonde that has been validated to provide data of useful quality.
Develop an assay based on trichloroethene labelled with Carbon 14 (^{14}C-TCE) that will allow for determination of TCE co-oxidation rates in groundwater samples.	Measurements of the rate of accumulation of ^{14}C label in transformation products of TCE in groundwater samples compared to accumulation in filter-sterilized controls. Accumulation of ^{14}C label in products measured with a scintillation counter.	Objectives are met if consumption rates for ^{14}C-TCE in groundwater samples are statistically significant in comparison to controls containing filter sterilized groundwater.	This performance metric was met in 8 of 19 samples that were evaluated. Out of the 19 groundwater samples evaluated, statistically significant rates of TCE co-oxidation were observed in eight (8).
Combined application of *q*PCR and EAP will show the presence of bacteria with active enzymes in groundwater.	Data on the abundance of bacteria in groundwater reacting to the EAP or that contain DNA that is amplified by the *q*PCR primers for oxygenase enzymes.	EAP and *q*PCR can be configured and implemented to provide sufficient sensitivity for application to diverse aerobic aquifers.	EAP and *q*PCR analyses were applied to 19 samples. Performance metrics met by showing presence and activity of TCE cometabolizing bacteria at numerous sites.
Compare consumption rates of ^{14}C-TCE to number of bacteria with active enzymes.	Rate constants for co-oxidation of TCE in water samples as determined by ^{14}C-TCE assay and data on abundance of bacteria reacting to the EAP or that contain DNA amplified by the *q*PCR primers.	In each sample, the abundance of bacteria responding to at least one of the EAP assays is above the quantitation limit and the abundance of DNA amplified by at least one qPCR assay is above the quantitation limit.	Cooxidation of TCE was only detected in 8 water samples where the rate constants for cooxidation were > 0.01 per year. The abundance of bacteria with active enzymes can only be used to evaluate sites where the rate constants are > 0.01 per year.

Table 3.2. Quantitative Performance Objectives

Objective(s)	Data Requirement(s)	Success Criteria *	Results
Quantify relationship between magnetic susceptibility from a direct-reading downhole sonde and that from laboratory analyses on samples from boreholes into which PVC monitoring wells were installed.	Magnetic susceptibility data from laboratory analyses of core samples from boreholes into which PVC wells were installed. A readily available magnetic susceptibility sonde (probe) was lowered into these same wells (Section 4), and real-time magnetic susceptibility data were collected.	The correlation between magnetic susceptibility determined using the sonde and that from laboratory analyses of core samples were determined. The Pearson's correlation coefficient, r, was calculated. If r is greater than 0.75, then the criteria for this performance objective has been met.	The plot of mass magnetic susceptibility from the sonde versus that determined from lab analyses of core samples yields $r = 0.94$ and $R^2 = 0.88$. Thus, this performance objective is met and the downhole sonde is considered to be a good tool for collecting representative magnetic susceptibility data from existing PVC wells.
Determine first order rates of TCE co-oxidation using a ^{14}C-TCE assay. Rate constants were determined by measuring the rate of accumulation of ^{14}C label in transformation products in water samples.	Data on the rate of accumulation of ^{14}C label in TCE transformation products as provided by the ^{14}C-TCE assay. Rate constants were determined in groundwater samples taken from 19 wells at five sites. There were four wells at each of four sites and three wells at one site.	Objective met if rates of ^{14}C product accumulation from ^{14}C-TCE in groundwater samples are statistically significant in comparison to controls containing filter-sterilized groundwater (FSGW) at 95% confidence.	This performance metric was met in 8 of 19 water samples. Out of the 19 groundwater samples evaluated, statistically significant rates of TCE co-oxidation were observed in 8, with first order rates ranging from 0.00658 to 2.65 yr^{-1}.
Quantify numbers of oxygenase genes present in groundwater community with *q*PCR analysis, and numbers of bacteria with active oxygenase enzymes can be quantified using EAP analysis.	Quantify activity of oxygenase genes based on EAP and surrogate *q*PCR measurements. Quantify activity measured for positive control organisms, negative controls, matrix spikes and blanks to determine specificity of EAP and *q*PCR. Quantify activity in replicate samples from same well.	EAP and *q*PCR techniques provide reproducible data when comparing groundwater replicates (<30% Relative Percent Difference [RPD]). Blanks have no background fluorescence. Positive controls show active enzymes. Matrix spikes provide 70 to 130% recovery of positive control organism. Control assays perform as expected.	In general, qPCR results corresponded to the EAP results for the qPCR Primer for Phenol Monooxygenase (Phenol Hydrolyase) Enzyme (PHE) and qPCR Primer for Ring-Hydroxylating Toluene Monooxygenease Enzyme (RMO) primer sets, but not for the qPCR Primer for Toluene Dioxygenase Enzyme (TOD) and qPCR Primer for Toluene/Xylene Side Chain Monooxygenase Enzyme (TOL) primer sets. Gene targets for qPCR Primer for Soluble Methane Monooxygenase Enzyme (sMMO) were only detected significant levels (>10^3 cells/ml) at three of the 19 wells tested.
Demonstrate ability to determine TCE transformation rates by numbers of bacteria with active co-oxidation enzymes determined by EAP and *q*PCR	Rate constants for co-oxidation of TCE in water samples as determined by the ^{14}C-TCE assay and data on the abundance of bacteria reacting to the EAP or that contain DNA that is amplified by the *q*PCR primers	The slope of a regression of the common logarithm of the rate constant for TCE co-oxidation on the common logarithm of the abundance of EAP or *q*PCR markers will be greater than zero at 95% confidence.	Slope greater than zero at 95% confidence for the trans-Cinnamonitrile (CINN) EAP and for the PHE, RMO, and MMO qPCR markers. The prediction interval of the regression was used to develop a screening approach to evaluate whether TCE co-oxidation might be useful for MNA at a site.

4.0 SITE DESCRIPTION

The following five (5) sites were selected for analysis under this program because there is an existing database for either the magnetic susceptibility of core samples from the site, or because there are existing data on the numbers of bacteria in groundwater that have enzymes that might degrade TCE, or both.

- Former Plattsburgh Air Force Base (AFB)
- The New Brighton/Arden Hills Superfund Site (TCAAP)
- Hill AFB Operable Unit 10
- Hopewell Junction
- Tooele Army Depot (TEAD)

The reader is referred to the Technical Report (ESTCP, 2017) for this project for detailed discussions for each of these Sites.

Former Plattsburgh AFB had existing data on magnetic susceptibility from core samples. Any potential contribution of aerobic biodegradation of the chlorinated solvents was not understood at the time the remedy was selected. An evaluation of the further contribution of aerobic co-oxidative biodegradation was not included in the last five-year review, but is completed under this investigation. In this study, the potential for abiotic TCE degradation was evaluated by measuring the magnetic susceptibility of aquifer sediment in two wells. The potential for cooxidation of TCE was evaluated in water samples from four wells.

The New Brighton/Arden Hills Superfund Site (TCAAP) is located on the north end of the former Twin Cities Army Ammunition Plant (TCAAP), in the city of Shoreview, Minnesota. Data on magnetic susceptibility from core samples existed for the Site. Any potential contribution of aerobic biodegradation of the chlorinated solvents was not understood at the time that the remedy was selected. An evaluation of the further contribution of aerobic co-oxidative biodegradation was not included in the last five-year review, but was completed under this investigation. In this study, the potential for abiotic TCE degradation was evaluated by measuring the magnetic susceptibility of aquifer sediment in three wells. The potential for cooxidation of TCE was evaluated in water samples from four wells.

Hill Air Force is a major U.S. AFB located in northern Utah, just south of the city of Ogden, near the towns of Clearfield, Riverdale, Roy, Sunset, and Layton. It is located about 30 miles north of Salt Lake City. Operable Unit 10 encompasses the Building 1200 Area along the western boundary of Hill AFB and extends off-base into the cities of Clearfield, Sunset, and Clinton. Industrial activities at the 1200 Area of OU 10 began in the early 1940s. A variety of chemicals, including chlorinated solvents such as PCE and TCE, were used in those activities. Most industrial activity in the 1200 Area ceased in 1959, and most of the 1200 Area buildings were remodeled for administrative functions. Currently, the majority of these buildings are still being used for administration purposes. Hill AFB Operable Unit 10 had data on EAPs but no data on mass magnetic susceptibility. In this study, the potential for abiotic TCE degradation was evaluated by measuring the magnetic susceptibility of aquifer sediment in three wells. The potential for cooxidation of TCE was evaluated in water samples from three wells.

Hopewell Junction, NY is located about 40 miles north of New York City. It is not a DOD site. It was included in this project because it has a unique database that allowed evaluation of the downhole magnetic susceptibility sonde. Borehole core samples were acquired that corresponded to the screened interval of 11 Environmental Protection Agency (EPA) monitoring wells and analyzed for magnetic susceptibility in the laboratory. A total of 68 subcores were analyzed for magnetic susceptibility using laboratory methods. Hopewell Junction also was evaluated using EAPs. However, it was not originally assayed using *q*PCR for DNA associated with oxygenase enzymes. In addition, before this project, aerobic biodegradation had not been incorporated in the site conceptual model at a quantitative level. This project includes EAPS, *q*PCR analyses, and a ^{14}C-TCE assay for this site, as discussed in Section 5 of the Technical Report. In this study, the potential for abiotic TCE degradation was evaluated by measuring the magnetic susceptibility of aquifer sediment in six wells. Three other wells were logged for the convenience of the Army Corps of Engineers. The potential for cooxidation of TCE was evaluated in water samples from four wells.

TEAD is a United States Army post located in Tooele County, Utah. It is located south of the city of Grantsville, southeast of the city of Erda and southwest of the city of Tooele. Magnetic susceptibility data were collected in numerous core samples during the installation of monitoring wells at the Site. Under this effort, the project team conducted downhole magnetic susceptibility analyses and compared the results to the laboratory-based analyses that are already available. Furthermore, in their 2013 Evaluation of MNA (Parsons, 2013), Parsons recommended using EAPs to evaluate aerobic co-oxidation of TCE. Under this effort, the project team will complete EAP analyses in the wells that were installed in the borings from which magnetic susceptibility analyses were completed. The team will also determine the rate constant for aerobic biodegradation of TCE in the groundwater. In this study, the potential for abiotic TCE degradation was evaluated by measuring the magnetic susceptibility of aquifer sediment in one well. The potential for cooxidation of TCE was evaluated in water samples from four wells.

In addition to the sites listed here, one other site was used for the magnetic susceptibility analysis, but no samples were collected for the ^{14}C assay or the EAP/*q*PCR analyses. Specifically, monitoring well U2-043 at OU-2 at Hill AFB was analyzed for mass magnetic susceptibility by collecting borehole core samples on December 7th and 8th 2015. Because the sampling team was so close to this Operable Unit during sampling at Hill AFB, OU-10, groundwater monitoring well U2-043 was sampled with the downhole sonde upon completion of sampling at OU-10. The sonde and borehole core laboratory analytical data from monitoring well U2-043 are included in the statistical analyses completed for this project.

5.0 TEST DESIGN

The test design developed for this project was designed to allow the efficacy of MNA to be evaluated more efficiently and accurately than it has been in the past using tools that had already been developed but had not been adequately tested for environmental applications. Specifically, the techniques described and quantified in this report benchmark abiotic degradation by magnetite and aerobic co-oxidation at sites contaminated with chlorinated ethylenes.

5.1 CONCEPTUAL EXPERIMENTAL DESIGN

In order to complete the objectives outlined for this project, seven (7) tasks were completed:

- Task 1 – Development of Demonstration Plan/Field Sampling Plan.
- Task 2 – Field Work - Deployment of Magnetic Susceptibility Sonde and Field Sampling for EAPs, *q*PCR Assays of Co-Oxidation Enzymes, and Direct Assay of Rate of Aerobic Biodegradation.
- Task 3 – Develop a Carbon 14 Tracer Procedure to Directly Assay the Rate of TCE Aerobic Biodegradation.
- Task 4 – Laboratory Work to Conduct Carbon 14 Tracer Direct Assay of the Rate of TCE Aerobic Biodegradation.
- Task 5 – Laboratory Work to Implement EAPs and the *q*PCR Assays for Oxygenase Enzymes that can Co-Oxidize TCE.
- Task 6 – Final Report.
- Task 7 –Project Management.

Each task is discussed in the following subsections.

5.1.1 Task 1 - Development of Demonstration Plan/Field Sampling Plan

Under this task, a Demonstration Plan that met all ESTCP requirements and guidelines was prepared and approved by ESTCP. This document included the objectives of the demonstration, qualitative and quantitative criteria for success, the methodologies to be employed, and the measurements that were required to determine success. The Demonstration Plan was Approved in April 2016.

5.1.2 Task 2 – Field Work - Deployment of Magnetic Susceptibility Sonde (Probe) and Field Sampling for EAPs, *q*PCR Assays of Co-Oxidation Enzymes, and Direct Assay of Rate of Aerobic Biodegradation

Task 2 consisted of:

1. Mobilization;
2. Collecting magnetic susceptibility data using a downhole sonde;
3. Collecting groundwater samples for submission to Clemson University and PNNL, and;
4. Demobilization.

For this task, Todd Wiedemeier and Dr. John Wilson traveled to the five sites described in Section 4 to deploy the magnetic susceptibility sonde and collect the data necessary to compare these data to those previously collected from soil cores at the sites. The task also was used to collect the water samples that were assayed using EAPs, *q*PCR for oxygenase enzymes, and for the rate of aerobic biodegradation of TCE using the ^{14}C-labeled TCE assay.

5.1.3 Task 3 - Develop a Carbon 14 Tracer Procedure to Directly Assay the Rate of TCE Aerobic Biodegradation

Task 3 was used to refine a method for purifying ^{14}C-TCE beyond what was achieved in previous studies. Two Gas Chromatograph (GC) columns in series was evaluated. It was determined that a single column provided an adequate level of purification. Although the target level for impurities was not met (i.e., less than 0.01% of the total ^{14}C added to the serum bottles), the level of impurities was sufficiently low to permit detection of first order co-oxidation rates as low as 0.00658 yr^{-1}. This necessitated increasing the incubation time of the assay to 40-46 days, to allow for sufficient accumulation of products. A potential concern with an incubation time longer than a few days is changes in the microbial community. However, as results for the groundwater samples indicate, the rates of ^{14}C product accumulation tended to diminish with time. If changes in the microbial community did occur, it resulted in a more conservative estimate of the rates of TCE degradation. Details regarding the approach that was used to accomplish Task 3 are provided in Appendix B.

5.1.4 Task 4 - Laboratory Work to Conduct Carbon 14 Tracer Direct Assay of the Rate of TCE Aerobic Biodegradation

The goal of Task 4 was to determine pseudo-first order rate constants for TCE co-oxidation in groundwater samples from five sites, with samples taken from four wells per site. The assay was performed in 160 mL clear, borosilicate glass serum bottles (Wheaton®; Boston round, 125 mL) that were cleaned, dried, and autoclaved along with Teflon-faced gray butyl rubber septa and aluminum crimp caps (20 min, 121 °C). The bottles were labeled and massed (balance precision, ± 0.01 g) and then shipped to Wiedemeier & Associates for use in sample collection. In the field, triplicate 100 mL groundwater samples were collected from each monitoring well. The serum bottles were immediately capped with their respective Teflon-faced gray butyl rubber septum and crimped with an aluminum cap. The bottles were stored on ice and shipped overnight to Clemson University. Upon arrival, the bottles were removed from the packaging and warmed to room temperature (22 ± 2°C), quiescently in the dark, for approximately 24 hours before addition of the ^{14}C-TCE stock solution.

For a given site, triplicate serum bottles were received from two wells on the first day, and a second set of triplicates was received from two additional wells on the following day. When the first set of triplicates groundwater samples was prepared, a set of triplicate distilled deionized water ((DDI) controls was prepared at the same time.

An overview of how the assay was performed is provided in Section 5.1.4.1 of ESTCP (2017).

5.1.5 Task 5 - Laboratory Work to Implement EAPs and the *q*PCR Assays for Oxygenase Enzymes that can Co-Oxidize TCE

PNNL assayed water samples for the density of bacteria that react to one of three EAPs. The probes determine if the bacteria express an oxygenase enzyme that can carry out aerobic co-oxidation of PCE or TCE. PNNL also used *q*PCR to assay water samples for the abundance of gene copies for selected oxygenase enzymes. PNNL assayed water from the same wells that were sampled for Task 4. This allowed a statistical comparison of the abundance of cells that respond to an EAP, or gene copies of oxygenase enzymes, to the directly measured rate of aerobic biodegradation.

5.1.6 Task 6 – Analyze Data, Validate Cost and Performance Data, and Prepare Final Report

This task represents what is presented in this report, which details the findings of the work described in the Demonstration Plan (ESTCP, 2016). The contribution of abiotic degradation and aerobic biodegradation at each site is evaluated herein. This is accomplished by comparing the rate constant for attenuation associated with abiotic degradation by magnetite in the aquifer matrix and the rate constant associated with aerobic co-oxidation to the bulk rate constant for natural attenuation at the site. If the rates of abiotic degradation by magnetite or the rates of aerobic biodegradation can meet the goals for MNA, this has the potential to save the DOD significant amounts of money in unnecessary remediation costs.

Project ER-201129 showed the relationship between magnetic susceptibility and degradation rate using magnetic susceptibility data from soil core samples. The material presented in this report shows that a relatively inexpensive downhole probe can be used for measurement of magnetic susceptibility in lieu of using soil core samples, the acquisition of which can be quite expensive at sites where intrusive site characterization activities have already been completed. This has the potential to save the DOD significant amounts of money in unnecessary drilling costs.

This report also evaluates the utility of EAPs and *q*PCR assays by comparing the rate constant for aerobic co-oxidation to the abundance of cells that react with EAPs or the abundance of genes copies for oxygenase enzymes. To allow extrapolation of this approach to other sites, this report provides protocols for collecting and analyzing magnetic susceptibility data, how to perform the ^{14}C-TCE assay and the EAP assay, and the procedures to submit samples for *q*PCR evaluation.

Cost and performance data are provided for down-hole determination of magnetic susceptibility, for the EAPs, for the *q*PCR assays, and for the direct assay of the rate constant for aerobic biodegradation. Cost and performance data are validated to determine if the performance objectives established in Section 3 have been met.

5.1.7 Task 7 – Project Management

This task was used for project management and project communication, and is a fairly common task. This task is described in detail in the Demonstration Plan (ESTCP, 2016).

5.2 BASELINE CHARACTERIZATION

Section 4 of ESTCP (2017) provides an in-depth discussion for the majority of baseline characterization activities. Baseline characterization was conducted for magnetic susceptibility using borehole core samples and fixed-base laboratory analyses for the sites discussed in Section 4 except Hill AFB OU-10. The results of baseline sampling for magnetic susceptibility using core samples and fixed-base laboratory analyses are summarized in Table 4.1.2 of ESTCP (2017).

Previous baseline characterization for EAP was completed for Hill AFB OU-10 and for the Hopewell Precision Site prior to the field work conducted under this effort, as described in Section 4 of ESTCP (2017). For Hill AFB, *q*PCR analyses were also performed. For the other sites, baseline for EAP and *q*PCR was established during this demonstration. No direct correlation between quantification of oxygenase enzymes to TCE transformation rates was performed prior to this work, so a baseline for this comparison is provided by this work.

The only additional baseline characterization that was required for this demonstration was development of the ^{14}C-TCE assay. Since the ^{14}C-TCE assay had not previously been deployed for the purposes proposed in this study, it was not possible to precisely define baseline activities. However, as described in ESTCP (2017; Section 3.2.2.2), a preliminary evaluation was performed with locally sourced water from a seep that presumptively contained a high level of dissolved organic matter. It was anticipated that the naturally occurring organics included aromatic compounds that would support the induction of oxygenases. A net rate coefficient of $2.37\times10^{-2}\pm 1.67\times10^{-2}$ yr^{-1} was determined, which gives a half-life of 29 yr (95% confidence interval = 17-99 yr). This result suggested that the ^{14}C assay is adequately sensitive to detect TCE co-oxidation in groundwater samples at rates that are meaningful for evaluating natural attenuation.

5.3 TREATABILITY OR LABORATORY STUDY RESULTS

This section provides the results of laboratory studies performed by Clemson University and PNNL. This section is sufficiently detailed to fully describe the results. For the work performed by Clemson University, detailed data sets are provided in appendices in ESTCP (2017). This Cost & Performance Report summarizes the laboratory study results.

5.3.1 Treatability or Laboratory Studies Performed by Clemson

The use of ^{14}C-labeled compounds to determine the rate at which a parent compound degrades, as well as the identity of the products formed, has been in practice for decades. This includes the fate of ^{14}C-TCE and other chlorinated organic contaminants. The Freedman laboratory has extensive experience in the use of ^{14}C-labeled compounds. Although there is an extensive collection of literature on experiments with ^{14}C-labeled environmental contaminants, it is noteworthy that the frequency of using ^{14}C has diminished over the last decade or so. As a consequence, many ^{14}C-labeled compounds such as TCE are no longer commercially available as a stock item. Custom synthesis is required to obtain the compound, including the ^{14}C-TCE used for this project.

The most common way for vendors to deliver ^{14}C-labeled compounds is dissolved in a solvent. For this project, the ^{14}C-TCE was purchased from Moravek Biochemicals dissolved in acetonitrile. This necessitated separating the TCE from the solvent. The same GC approach described in previous research (Darlington et al., 2008, 2013) was used; this also resulted in an increase in the purity of the ^{14}C-TCE added to groundwater samples.

An important feature of the assay developed for this project in comparison to past work is that water samples were repeatedly removed from the same serum bottles over time, in order to measure the accumulation of ^{14}C-products. The size of the aqueous samples (3.1 mL) was large enough so that it became necessary to take into account the ^{14}C activity removed with each sample. Consequently, a mass balance model was needed in order to account for both the ^{14}C products that accumulated and the ^{14}C removed during sampling. Fitting of the ^{14}C product data to the mass balance model permitted determination of the pseudo first order rate coefficient. In the previous studies, the distribution of ^{14}C was determined using the entire contents of a serum bottle. As such, in order to determine a rate, it was necessary to sacrifice replicate bottles over time. This was not feasible for the current project, due to the large number of bottles that would have needed to be prepared. This led to development of an assay that allowed for repeat sampling from the same bottle.

5.3.2 Treatability or Laboratory Studies Performed by PNNL

Quantitative polymerase chain reaction provides evidence for the presence of cometabolism genes in groundwater samples, while EAP provided lines of evidence that there are active cometabolic enzymes in a groundwater sample. Groundwater from five sites across the U.S. were analyzed using qPCR and EAP, and surprisingly few of the samples showed the presence and activity of the cometabolic oxygenase enzymes probed for during the project. Four of the 19 wells analyzed using the phenylacetylene (PA) and 3-hydroxyphenylacetylene (3HPA) EAP, showed activity considered to be statistically significant (>8x103 cells/ml). Cinn only showed positive results for two of the nineteen wells tested.

In general, qPCR results corresponded to the EAP results for the qPCR Primer for Phenol Monooxygenase (Phenol Hydrolyase) Enzyme (PHE) and qPCR Primer for Ring-Hydroxylating Toluene Monooxygenease Enzyme (RMO) primer sets, but not for the qPCR Primer for Toluene Dioxygenase Enzyme (TOD) and qPCR Primer for Toluene/Xylene Side Chain Monooxygenase Enzyme (TOL) primer sets. Gene targets for qPCR Primer for Soluble Methane Monooxygenase Enzyme (SMMO) were only detected significant levels (>103 cells/ml) at three of the 19 wells tested.

5.4 FIELD TESTING

Field testing consisted of the collection of mass magnetic susceptibility data for the aquifer matrix using a downhole sonde and collection of groundwater samples for the ^{14}C TCE assay, EAPS, and *q*PCR. Magnetic susceptibility versus depth data were collected using the downhole sonde in monitoring wells that were installed in boreholes from which magnetic susceptibility data were collected from soil borehole core data and analyzed in a fixed-base analytical laboratory.

5.5 SAMPLING METHODS

5.5.1 Magnetic Susceptibility Sampling

Figure 5.5.1 shows the exterior of the magnetic susceptibility sonde. This figure also includes probe dimensions and operating parameters. As can be seen from this figure, the magnetic susceptibility sonde has a diameter of 45 mm (1.77 inches).

Probe Parameters

Diameter	45 mm
Length	125 cm
Weight	4.5 kg
Max. Working Temperature/Pressure	75°C/20 MPa
Min. No. of Cable Conductors	2
Supply Voltage Range	30 – 45 V_{DC}
Max./Nominal Current Consumption	100/40 mA_{DC}
Supply Voltage Polarity	+ on central cond.
	– on probe casing

Measuring Parameters

Sensor	four coil system
Intercoil Spacing 1. channel	25 cm
Intercoil Spacing 2. channel	30 cm
Operating Frequency	≈2 kHz
Communication (negative pulse)	0 – 20 000 cps
Measuring range	10^{-5} – 0.5 SI units
	10^{-4} – 2 SI units
Accuracy	< 3% F.S.
Zero Drift	< 2 10^{-5} SI units/10°C

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Figure 5.5.1. W&R Instruments HM-453S Borehole Magnetic Susceptibility Sonde with Dimensions.

For the sonde used in this study, the W&R Instruments HM-453S, the Tx-Rx spacing is 25 cm (9.84 inches). This equates to optimal readings being made at roughly 20 cm (~8 inches) from the sonde (perpendicular), with the accuracy of the magnetic susceptibility measurements dropping off beyond this distance. Based on this, boreholes less than about 40 cm (~16 inches) in diameter are optimal for the HM-453S. Magnetic susceptibility readings made in boreholes larger than this will not be as accurate as those made in smaller boreholes.

The sonde was recalibrated immediately prior to use in each monitoring well. The sonde was first calibrated against air, which was taken to have a value of 0, and then against a standard that was equivalent to a magnetic susceptibility of 2.94E-6 m^3/kg. After calibration, the sonde was introduced into either 5.1 cm (2 inch) or 10.2 cm (4 inch) inner diameter Poly(Vinyl Chloride) (PVC) groundwater monitoring wells using a Mount Sopris Instruments R-4200-1000-200 Mini Winch (Figure 5.5.2). Data from the sonde were recorded using the Mount Sopris Instruments R-5MXA-1000 Matrix Console (Figure 5.5.2). The sonde is capable of taking measurements when it is being lowered into, or raised from, a monitoring well. The sonde was lowered or raised at a rate of about 9 feet per minute, and magnetic susceptibility measurements were collected about every 1.3 seconds. This equates to about 8.7 readings being taken for every foot the sonde travels down or up the borehole. The sonde was not centered in the well or intentionally forced against the sidewall as it was introduced into the well. The magnetic susceptibility sonde was found to fit down both 2-inch and 4-inch PVC monitoring wells. However, if a 2-inch PVC monitoring well was compromised, such as with uneven joints or casing that was not sufficiently straight, then it was problematic to get this sonde into the well.

The components required to collect downhole data using a magnetic susceptibility sonde such as that shown in Figure 5.5.1 include (Figures 5.5.2 and 5.5.3):

1) Sonde with coaxial cable.
2) Tripod with pulley to guide the wireline cable with the sonde attached down the well.
3) Winch.
4) Data recorder to record and reduce data.
5) WellCAD or similar to analyze and display data.

Figure 5.5.2 shows the data logger, winch, and tripod (various configurations) used to lower the magnetic susceptibility sonde into a monitoring well to collect magnetic susceptibility data. The data logger is attached directly to the winch and is then connected to a computer. The tripod and attached pulley are situated over the well and the sonde is slowly lowered into the well using the winch. A 1000-watt invertor generator was found to be sufficient for this configuration, with the sonde being lowered to depths as great as 450 feet below ground surface (bgs). As with any downhole equipment, the magnetic susceptibility sonde was thoroughly decontaminated between each well, as described in ESTCP (2016).

Figure 5.5.2. Data Logger, Winch and Tripod with Pulley for Magnetic Susceptibility Sonde

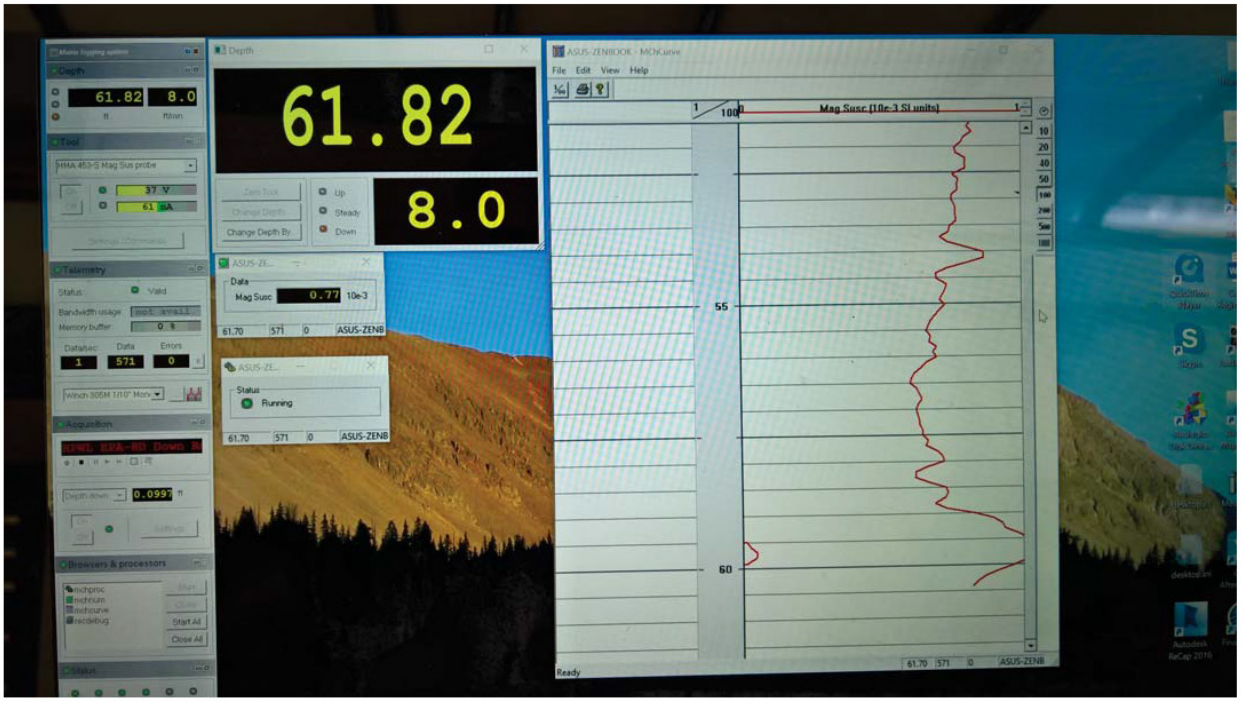

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Figure 5.5.3. Real-Time Data Readout During Logging with a Magnetic Susceptibility Sonde

5.5.2 Groundwater Sampling for ^{14}C TCE Assay, EAPS, and qPCR

Special care was taken to prevent contamination of groundwater samples. The two primary ways in which sample contamination can occur are through contact with improperly cleaned equipment and by cross-contamination through insufficient decontamination of equipment between wells. To prevent such contamination, all non-dedicated down-hole equipment was thoroughly cleaned before and after field use and between uses at different sampling locations according to the procedures presented in ESTCP (2016). In addition to the use of properly cleaned equipment, a clean pair of new, disposable nitrile gloves was worn each time a different well was sampled. Dedicated polyethylene, and in many cases, silicone tubing was used for the pumps used for this effort. This tubing was disposed of between each well. This tubing was always stored away from any substances that could cause contamination. Wells were sampled sequentially from areas suspected to be least contaminated to areas suspected to be more contaminated.

Prior to removing any water from monitoring wells, the static water level was measured. An electrical water level probe was used to measure the depth to groundwater below the datum to the nearest 0.01 foot. None of the wells sampled had nonaqueous-phase liquid (NAPL).

Prior to sample collection, groundwater was withdrawn from the well using a peristaltic pump, a Grundfos Redi-Flo II® pump, a weighted disposable bailer, or a Hydrasleeve™. The Hydrasleeve™ was used only to collect samples at Tooele Army Ammunition Depot because of groundwater disposal requirements and because of the depth to groundwater.

The Grundfos pump was used only for sampling in OU10-019. Purging and sample collection was performed at 19 monitoring wells (ESTCP, 2017). Where possible, field measurements for dissolved oxygen (DO), ORP, potential of Hydrogen (pH), temperature, specific conductance were collected during well purging using a flow through cell. In addition, field measurements for Fe(II) concentration were made immediately after well purging using a Hach DR 890 Colorimeter. Laboratory samples were collected immediately following well purging.

Water from sampling devices was directly discharged into the sample container. The water was carefully poured down the inner walls of the sample bottle to minimize aeration of the sample. Four sample acquisition techniques were used to collect groundwater samples, including:

1) Use of a peristaltic pump with tubing placed directly into the well (majority of wells);
2) Use of a Grundfos submersible pump (one well, U10-019);
3) Use of a bailer and draining the groundwater out of the base of the bailer (well MW-02-006 at the former Plattsburgh AFB), and;
4) Use of a Hyrdasleeve™ in conjunction with a peristaltic pump. The Hydrasleeve™ was lowered into the well to collect the water sample, raised to the surface and temporarily hung from the lip of the well. A peristaltic pump was then used to remove water from the Hydrasleeve™. This technique was used for all of the wells sampled at Tooele Army Ammunition Depot because no purge water could be generated.

Sample containers were filled as follows:

1) For samples sent to PNNL for EAP analyses, the sample containers were filled so that no air space (headspace) remained in the container, and;
2) For samples sent to Dr. Freedman's lab at Clemson, 160 mL serum bottles were filled with approximately 100mL of groundwater and sealed with a crimp cap with a Teflon septum.

Sample containers and appropriate container lids and labels were provided by the analytical laboratories at Clemson University and PNNL.

No chemical preservatives were added to the sample containers for the samples sent to Clemson University or PNNL. Samples were properly prepared for transportation to the laboratory by placing the samples in a cooler containing ice to maintain a shipping temperature of 4°C.

5.6 SAMPLING RESULTS

5.6.1 Magnetic Susceptibility Sonde

An easy to use approach for measuring magnetic susceptibility in existing non-metallic groundwater monitoring wells was developed under this program. Users are able to easily obtain accurate magnetic susceptibility data using a relatively-inexpensive commercially-available downhole sonde. In order to make sure that the downhole sonde provides reliable results, the relationship between magnetic susceptibility from the direct-reading downhole sonde was compared to that from laboratory analyses on samples from boreholes into which PVC monitoring wells were subsequently installed. The correlation between magnetic susceptibility determined using the sonde and that from laboratory analyses of core samples was determined by

preparing a plot of the magnetic susceptibility obtained from the sonde versus that obtained from laboratory analyses of soil borehole data. The Pearson's correlation coefficient, r, was then calculated. If r was greater than 0.75, then the criteria for this performance objective was considered to have been met. The plot of mass magnetic susceptibility from the sonde versus that determined from lab analyses of core samples yields r = 0.94 (R^2 = 0.88). Thus, the downhole sonde is considered to be a good tool for collecting representative magnetic susceptibility data from existing PVC wells.

Figure 5.6.1 compares the mean of the mass magnetic susceptibility obtained from the HM-453S sonde to the mean that was obtained from core samples. At each well location, the mean for the sonde data and the mean for the core samples were calculated from data collected over the same depth interval. The error bars in Figure 5.6.1 are the 95% confidence interval on the means. The Pearson Correlation Coefficient between the core means and the sonde means was r = 0.94.

If appropriate monitoring wells are available, downhole magnetic susceptibility sondes in groundwater monitoring wells can provide a less expensive alternative to the collection and analysis of borehole core data, and can provide data that can be used to evaluate field-scale rate constants for abiotic degradation of PCE, TCE, and *c*DCE by magnetite.

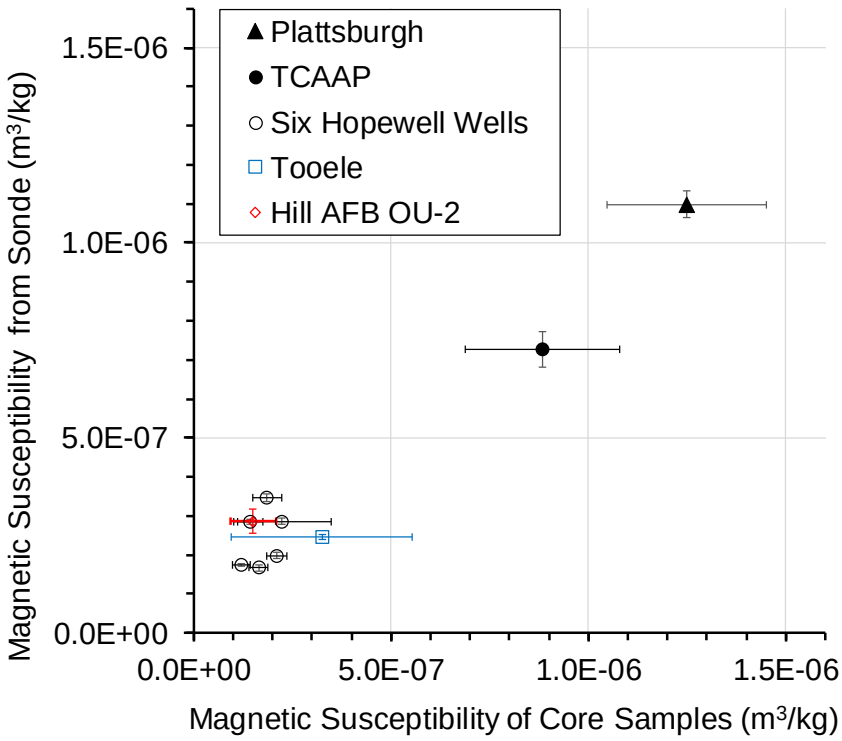

Figure 5.6.1. Relationship between Magnetic Susceptibility from Core Data and the Downhole Sonde.

Each data point is the mean of data from an individual well. The error bars are the 95% confidence interval on the mean.

Notice that the error bars are much narrower on the means of the sonde data (Figure 5.6.1). The confidence intervals are calculated from the standard error of the mean, which is the standard deviation of the samples divided by the square root of the number of samples. The sonde provided many more data points to contribute to the average. However, it is important to distinguish precision from accuracy. We had no independent standard to evaluate the accuracy of the sonde compared to the accuracy of the laboratory analyses.

In general, the means of the data from the sonde were in good agreement with the means of the data from the core samples. Table 5.1 compares the mean of the sonde analyses for each monitoring well to the mean of the core sample analyses. The means were compared with a t-test for the difference of means with unequal variance. For six of the ten wells, the test failed to reject the null hypothesis that there was no difference in the means at 95% confidence ($P > 0.05$). At four of the wells the means were different at 95% confidence. However, the mean of the sonde analyses varied from the mean of the core sample analyses by less than a factor of two (Table 5.1). The variation between data reported by the sonde and the laboratory analysis of core samples is acceptable for the purpose of evaluating a site for abiotic degradation of TCE.

The wells at the Hopewell site, OU-2 at Hill AFB, and at Tooele were 4-inch internal diameter (ID). The wells at TCAAP and Plattsburgh were 2-inch ID. The larger wells would have more air in the annular space, and would be more likely to have a greater radius of engineered sand pack between the wall of the bore and the screen or casing. This should tend to reduce the response in the sonde. Despite this expectation, the ratio of the response of the sonde to the core samples was generally higher in the 4-inch wells compared to the 2-inch wells (Table 5.1). The well at Tooele was the only exception. In the wells in the survey, there was no indication of a systematic bias in the magnetic susceptibility reported by the sonde in 4-inch ID wells.

Table 5.1. Comparison of Estimates of Mass Magnetic Susceptibility from a Downhole Sonde to Estimates from Laboratory Analysis of Core Samples

Location	Well	Mean of Magnetic Susceptibility		Ratio of Means	Number of Values in Mean		P*
		m^3kg^{-1}		Sonde/Cores			
		Sonde	Cores		Sonde	Cores	
Hopewell	EPA 19	1.8E-07	1.2E-07	1.44	71	6	1.8E-03
Hill OU-2	OU2-043	2.7E-07	1.4E-07	1.91	58	10	4.7E-05
Hopewell	EPA 16	2.8E-07	1.4E-07	1.96	273	8	7.4E-06
Hopewell	EPA 12D	1.7E-07	1.6E-07	1.03	223	15	0.65
Hopewell	EPA 10D	3.5E-07	1.9E-07	1.86	258	16	9.3E-08
Hopewell	EPA 15D	2.0E-07	2.1E-07	0.95	82	8	0.38
Hopewell	EPA 21	2.9E-07	2.2E-07	1.28	204	11	0.29
Tooele	D-23	2.2E-07	2.6E-07	0.76	261	8	0.44
TCAAP	O1U108	7.3E-07	8.8E-07	0.82	22	12	0.11
Plattsburgh	MW-02-030	1.2E-06	1.2E-06	0.95	302	18	0.51

*Probability of error, two tailed.

Figure 5.6.2 shows the relationship between first order rate constant for abiotic degradation and the magnetic susceptibility of aquifer materials. This figure is modified from ESTCP (2015) and He (2009) with additional data collected during this work. Figure 5.6.2 compares the first-order rate constant for degradation of chlorinated ethenes in unconsolidated aquifer sediments to the mass magnetic susceptibility of the sediment. Panel (a) of Figure 5.6.2 compares field-scale rate constants for removal of PCE, TCE, DCE, or VC. The rate constants were extracted from monitoring data at seven sites. Depending on the site, each data point may represent a rate constant for an individual chlorinated alkene, or it may represent a composite rate constant for PCE, TCE and DCE.

The dashed lines in Panel (a) of Figure 5.6.2 are prediction intervals on a new observation. The 95% prediction interval is an order of magnitude wide. This may reflect error and uncertainty in the estimates of the rate constants; however, this may also reflect true variation in the rate constants from one site to another. Lee and Batchelor (2002) noted in their laboratory experiments that adding 42.6 mM Fe(II) to a suspension of magnetite increased the rate constant for degradation of *c*DCE and VC by an order of magnitude. Iron(II) was present in groundwater at some of the sites depicted in Panel (a) of Figure 5.6.2, but not in others.

The rate constants in Panel (a) of Figure 5.6.2 are bulk rate constants and may include aerobic biodegradation of DCE in addition to abiotic degradation by magnetite. Panel (b) of Figure 5.6.2 summarizes a series of microcosm studies that are reported in He et al. (2009). The sediment used to construct the microcosms was autoclaved to kill any microorganisms. The rate constants for removal of the chlorinated alkenes in the microcosms can be safely attributed to degradation by magnetite. Only one of the sites used to extract field-scale rate constants was used for microcosm studies (TCAAP). Nevertheless, the range of field-scale rate constants and the range of rate constants in the microcosm studies were similar. There is no evidence that the field-scale rate constants are substantially faster than the rate constants from the microcosms studies.

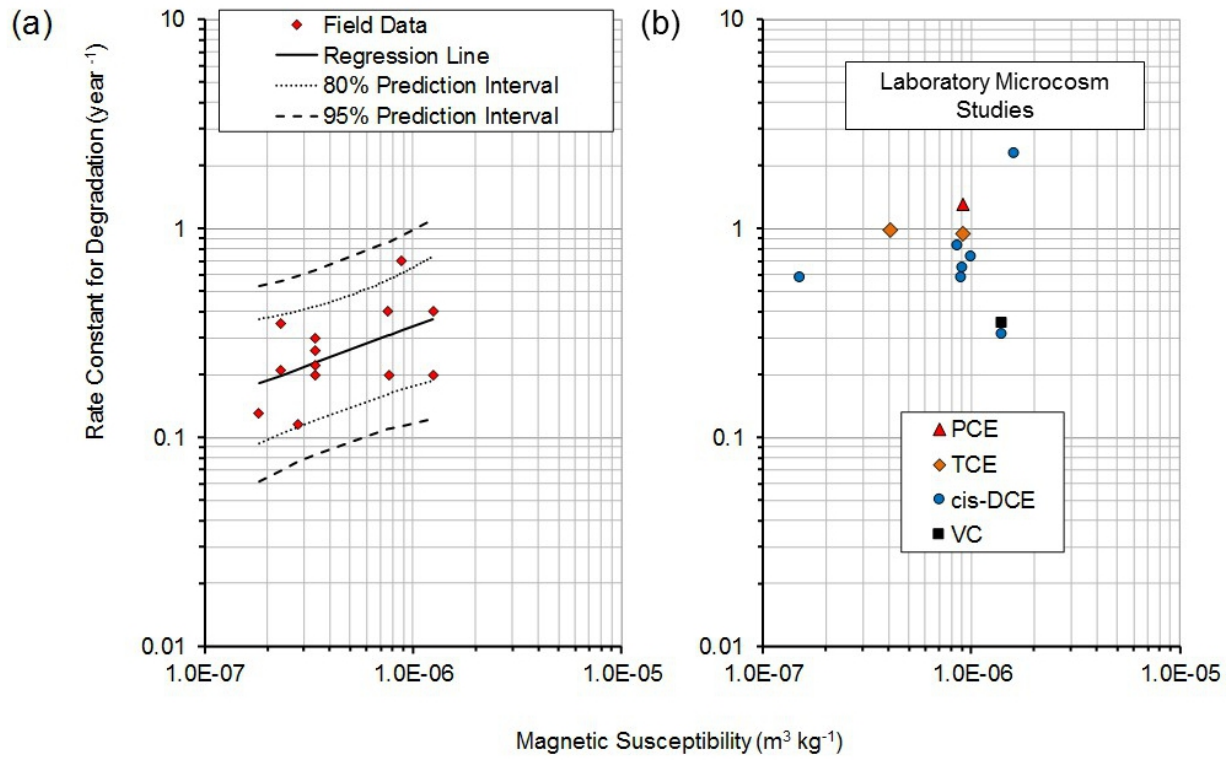

Figure 5.6.2. Relationship between First Order Rate Constant for Abiotic Degradation and the Magnetic Susceptibility of Aquifer Materials (modified from ESTCP [2015] and He [2009]).

5.6.2 Sampling Results for Data Generated by ^{14}C-Labeled TCE Assay

The ^{14}C-TCE assay allowed for quantification of pseudo first order rate constants in groundwater samples from eight of the 19 wells evaluated, at rates ranging from 0.00658 to 2.65 yr^{-1}. This translates to half-lives of 0.26 to 105 year (yr.) In groundwater from the other 11 wells, the rate of ^{14}C product accumulation was not statistically different from the filter-sterilized groundwater (FSGW) controls, so that no rate is reported. Although only a single GC column was used for purification of the ^{14}C-TCE, the level of impurities delivered to the serum bottles was sufficiently low to allow for detection of a half-life as long as 105 yr. This was due in part to extension of the incubation period from a few days to as long as 46 days, which permitted accumulation of a sufficient level of ^{14}C products to be distinguishable from the controls.

The initial plan was to use DDI water as the negative control. It was determined, however, that FSGW is more appropriate for this purpose. The rate of ^{14}C product accumulation in FSGW controls was statistically lower than in DDI water, likely due to the presence of constitutes that quench the autoradiolysis associated with decay of ^{14}C-TCE.

$^{14}CO_2$ constituted the majority of the ^{14}C product quantified, followed by Nonstrippable Residue labelled with Carbon 14 (^{14}C-NSR). This indicated that the groundwater samples that exhibited co-oxidation of TCE contained microbes with the ability to mineralize the products formed from the initial oxygenase attack on the compound.

5.6.3 Sampling Results for EAP and *q*PCR

Table 5.2 compares the geometric mean of the abundance of cells reacting to the three EAPs and the abundance of DNA amplified by the seven *q*PCR primers in the eight wells where co-oxidation of TCE was detected. The abundance of cells that reacted with the PA and 3-HPA probes was essentially identical. The abundance of cells that reacted to the Cinn probe may have been less, but any difference was not significant at 95% confidence.

No differences between the abundance of DNA amplified by the PHE primer, the RMO primer, the qPCR Primer for Ring-Hydroxylating Toluene Monooxygenease Enzyme (RDEG) primer and the qPCR Primer for Soluble Methane Monooxygenase Enzyme (mmoZ) primer could be distinguished that were significant at 95% confidence, and no differences between the abundance of DNA amplified by these primers and the abundance of cells that reacted with the EAPs could be distinguished at 95% confidence. It is likely that the Probes and the primers were interacting with the same population of cells. If this is true, then much of the DNA amplified by the primers is associated with living cells that are capable of supporting the activity of an oxygenase enzyme. This indicates that the qPCR primer sets amplified DNA that was associated with enzymes that were actually expressed in the cells that could cooxidize TCE.

There is no explanation for the behavior of the bacteria in the eleven wells where the cells reacted with the EAPs, but did not co-oxidize TCE at detectable rates. If the cells would react with the Probe, then why did they not react with TCE? It is possible that the cells were in a dormant state in the groundwater, and the presence of the Probe, or some other factor in the performance of the assay, restored the cells to an active state. This phenomenon will require further study before the behavior can be explained.

The abundance of DNA that was amplified by the TOD and TOL primers was orders of magnitude lower than the abundance of DNA amplified by the PHE primer, the RMO primer, the RDEG primer and the mmoZ primer. It is unlikely that enzymes coded by DNA amplified by the TOD and TOL primers are responsible for a significant fraction of TCE co-oxidation.

Table 5.2. Relative Abundance of Markers in the Eight Wells where TCE Co-oxidation Was Detected

Marker	Analyzed by	Geometric Mean	Lower 95% Confidence Interval	Upper 95% Confidence Interval	Number of Wells where DNA was Detected	Number of Wells where mRNA was Detected
		Gene Copy/ mL	Gene Copy/ mL	Gene Copy/mL		
PA	PNNL	2,700	820	9,100		
3-HPA	PNNL	2,700	1,000	7,000		
CINN	PNNL	1,200	280	4,800		
PHE	MI	950	69	1,300	8	3
PHE	PNNL	1,400	300	6000	8	
RMO	MI	1,800	140	24,000	4	0
RMO	PNNL	2,600	500	14,000	8	
RDEG	MI	790	103	6,000	8	1
sMMO	MI	210	37	1,200	8	2
mmoZ	PNNL	3,700	540	26,000	8	
TOD	MI	110	29	430	5	1
TOD	PNNL	16	3.8	67	8	
TOL	MI	280	40	2,000	3	0
TOL	PNNL	0.34	0.05	2.5	8	

Assays for DNA amplified by PHE primer, the RMO primer, the RDEG primer are commercially available. The PHE primer may be the best primer to describe TCE co-oxidation. In addition to a high abundance of DNA amplified by this primer, there was a reasonably high abundance of mRNA that was amplified by this primer.

Table 5.3 compares the slope of the regression line for the three EAP markers and the *q*PCR markers. According to the criterion in Table 3.2, a marker is useful to predict the value of the rate constant when the slope of the regression of the logarithm of the rate constant on the logarithm of the abundance of the marker is greater than zero at 95% confidence. The Cinn EAP marker met this criterion, though just barely, but the pH and 3-HPA EAP markers did not. The PHE, RMO, and MMO primers analyzed by both PNNL and MI met the criterion. The TOD marker as analyzed by PNNL met the criterion, but the TOD marker as analyzed by MI did not. This difference due to the greater sensitivity of the analyses performed by PNNL.

The TOL marker as analyzed by either PNNL or MI did not meet the criterion.

Table 5.3. Relative Abundance of Markers in the Eight Wells Where TCE Co-oxidation Was Detected

Marker	Analyzed by	Slope of Regression Line	Lower 80% Confidence Interval	Upper 80% Confidence Interval	Lower 95% Confidence Interval	Upper 95% Confidence Interval	Number of Wells in Regression
PA	PNNL	0.91	0.30	1.53	-0.13	1.95	8
3-HPA	PNNL	1.04	0.23	1.85	-0.34	2.42	8
CINN	PNNL	0.86	0.39	1.33	**0.06**	**1.66**	8
PHE	MI	0.55	0.36	0.74	**0.22**	**0.87**	8
PHE	PNNL	0.90	0.47	1.31	**0.18**	**1.61**	8
RMO	MI	1.05	0.63	1.45	**0.10**	**1.98**	4
RMO	PNNL	0.81	0.48	1.12	**0.26**	**1.35**	8
RDEG	MI	0.68	0.40	0.95	**0.21**	**1.14**	8
sMMO	MI	0.70	0.33	1.08	**0.07**	**1.34**	8
mmoZ	PNNL	0.71	0.43	1.00	**0.23**	**1.20**	8
TOD	MI	1.40	0.19	2.59	-0.94	3.72	5
TOD	PNNL	0.94	0.56	1.33	**0.29**	**1.60**	8
TOL	MI	-0.10	-6.76	6.56	-27.59	27.39	3
TOL	PNNL	0.57	0.21	0.93	-0.04	1.18	8

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6.0 PERFORMANCE ASSESSMENT

Qualitative and quantitative performance metrics were initially established and performance assessed through project execution. Performance was assessed using the performance objectives listed in Section 3 as a benchmark. The following subsections relate to the results that pertain to these metrics and goals.

6.1 QUALITATIVE PERFORMANCE OBJECTIVES

This section discusses the qualitative performance objectives for this project, which are summarized in Table 3.1.

6.1.1 Develop an Easy to Use Procedure for Collecting Magnetic Susceptibility Data

To be able to readily assess abiotic degradation by magnetite, reliable, yet relatively inexpensive magnetic susceptibility data for the aquifer matrix along the solute flowpath must be available. In order to achieve this performance objective, the implementation of a magnetic susceptibility sonde at various depths and in various conditions was tested. Specifically, the field testing of existing, readily-available technology to quantify magnetic susceptibility in existing PVC monitoring wells using a commercially-available magnetic susceptibility sonde was conducted to determine ease of use and accuracy. This performance objective was met for the following reasons:

1) Based on the experience of the field crew (i.e., Mr. Wiedemeier and Dr. Wilson), the sonde was very easy to use. After the initial learning curve, which will be much reduced for people reading this document, the field crew was able to set up and have the sonde down-hole and ready to obtain measurements within 0.5 hour of arriving at a given sampling location. Of course, this assumes that the field crew has no problems gaining access to the sampling point.

2) Based on the data and discussion presented in Section 5.6.1 and ESTCP (2017), the sonde used for this project produces accurate magnetic susceptibility data.

6.1.2 Develop an Assay Based on ^{14}C-TCE That Will Allow TCE Co-Oxidation Rates in Groundwater Samples

The ^{14}C-TCE assay allowed for quantification of pseudo first order rate constants in groundwater samples from eight of the 19 wells evaluated, at rates ranging from 0.00658 to 2.65 yr^{-1}. This translates to half-lives of 0.26 to 105 yr. In groundwater from the other 11 wells, the rate of ^{14}C product accumulation was not statistically different from the FSGW controls, so that no rate is reported. Although only a single GC column was used for purification of the ^{14}C-TCE, the level of impurities delivered to the serum bottles was sufficiently low to allow for detection of a half-life as long as 105 yr. This was due in part to extension of the incubation period from a few days to as long as 46 days, which permitted accumulation of a sufficient level of ^{14}C products to be distinguishable from the controls.

In several of the groundwater samples, Volatile Organic Compounds (VOCs) were detected in addition to TCE. These decreased in amount during the incubation period, often at a faster rate than the TCE. It is not yet known if these co-contaminants contributed to co-oxidation of TCE.

The fact that co-oxidation occurred in some of the groundwater samples that did not contain VOCs other than TCE indicates their presence is not a requirement. Demonstration of co-oxidation in a surface water sample obtained from a seep with no prior exposure to chlorinated contaminants indicated that naturally occurring processes can support TCE co-oxidation at a meaningful rate.

The initial plan was to use DDI water as the negative control for the ^{14}C-TCE assay. It was determined, however, that FSGW is more appropriate for this purpose. The rate of ^{14}C product accumulation in FSGW controls was statistically lower than in DDI water, likely due to the presence of constitutes that quench the autoradiolysis associated with decay of ^{14}C-TCE.

A propanotrophic culture that co-oxidized TCE was used as a positive control to evaluate the ^{14}C-TCE assay. ENV485 was used to assess the effect of storage conditions on the co-oxidation rate. It was determined that handling the culture in the same manner as the groundwater samples (i.e., 24 hours (h) at 4 °C to mimic shipping on ice, followed warming overnight to room temperature) caused a modest decrease in the first order rate coefficient. This suggests that the co-oxidation rates for the groundwater are conservative with respect to in situ conditions.

6.1.3 Methods for Identifying Presence and Activity of Co-Metabolic Bacteria for TCE Oxidation

Results from the experiments demonstrated that both *q*PCR and EAP performed as expected for analysis of cometabolism genes in groundwater samples. While responses from most wells was considered low, the *q*PCR primer sets and EAP provided lines of evidence that there are active cometabolic enzymes in a groundwater sample.

In general, *q*PCR results corresponded to the EAP results for the PHE and RMO primer sets, but not for the TOD and TOL primer sets.

6.1.4 Demonstrate Baseline Method for Linking TCE Transformation Rates to Numbers of Bacteria with Co-Oxidation Enzymes

The ^{13}C-TCE assay was an effective tool to determine rate constants for TCE co-oxidation at some sites, but not at others. At 95% confidence, the ^{14}C-TCE assay could extract a rate constants for TCE co-oxidation from 8 of 19 water samples. The rate constant for total degradation of TCE is an upper boundary on the rate constant for biological co-oxidation. The highest rate constant in any well where biological co-oxidation could not be distinguished from radiolysis was 0.084 per year. This corresponds to a half-life of 8.3 years. At many sites, a rate constant of 0.084 per year would be make biological co-oxidation a plausible mechanism for a MNA remedy. The ^{13}C-TCE assay will provide a rate constant for TCE co-oxidation at may many sites where co-oxidation is a plausible remedy, but not all of them.

In every well sampled, whether TCE co-oxidation was detected or not detected, the abundance of cells reacting to each of the EAPs was above the quantitation limit. In every well sampled, the abundance of DNA that was amplified by the PHE, RMO, and MMO primers was above the quantitation limit.

6.2 QUANTITATIVE PERFORMANCE OBJECTIVES

This section discusses the qualitative performance objectives for this project, which are summarized in Table 3.2.

6.2.1 Evaluate the Accuracy of Data for Magnetic Susceptibility

To verify the validity of the data collected using the magnetic susceptibility sonde, the sonde was deployed in wells where soil samples were previously collected from soil borings prior to well installation and analyzed for mass magnetic susceptibility in an analytical laboratory. The data collected using the downhole sonde were then compared to the previously-collected borehole soil data.

The correlation between magnetic susceptibility determined using the sonde and magnetic susceptibility data collected using laboratory analysis of soil/sediment data was determined (Figure 5.6.1). The Pearson's correlation coefficient, r, was calculated and determined to be $r = 0.94$ ($R^2 = 0.88$). Based on the quantitative performance objective for this task of a Pearson's correlation coefficient greater than 0.75 ($r > 0.75$), this performance objective is considered met. Thus, the magnetic susceptibility sonde provides a good tool for collecting representative magnetic susceptibility data from existing non-metallic (e.g., PVC) monitoring wells.

Based on this, if appropriate monitoring wells are available, downhole magnetic susceptibility sondes in groundwater monitoring wells can provide a less expensive alternative to the collection and analysis of borehole core data, and can provide data that can be used to evaluate field-scale rate constants for abiotic degradation of PCE, TCE, and *c*DCE by magnetite.

6.2.2 Determine First-Order Rates of TCE Co-Oxidation Using a ^{14}C-TCE Assay

The ^{14}C-TCE assay allowed for quantification of pseudo first order rate constants in groundwater samples from eight of the 19 wells evaluated, at rates ranging from 0.00658 to 2.65 yr^{-1}. This translates to half-lives of 0.26 to 105 yr. In groundwater from the other 11 wells, the rate of ^{14}C product accumulation was not statistically different from the FSGW controls, so that no rate is reported. Although only a single GC column was used for purification of the ^{14}C-TCE, the level of impurities delivered to the serum bottles was sufficiently low to allow for detection of a half-life as long as 105 yr. This was due in part to extension of the incubation period from a few days to as long as 46 days, which permitted accumulation of a sufficient level of ^{14}C products to be distinguishable from the controls.

In several of the groundwater samples, VOCs were detected in addition to TCE. These decreased in amount during the incubation period, often at a faster rate than the TCE. It is not yet known if these co-contaminants contributed to co-oxidation of TCE. The fact that co-oxidation occurred in some of the groundwater samples that did not contain VOCs other than TCE indicates their presence is not a requirement. Demonstration of co-oxidation in a surface water sample obtained from a seep with no prior exposure to chlorinated contaminants indicated that naturally occurring processes can support TCE co-oxidation at a meaningful rate.

The initial plan was to use DDI water as the negative control for the ^{14}C-TCE assay. It was determined, however, that FSGW is more appropriate for this purpose. The rate of ^{14}C product accumulation in FSGW controls was statistically lower than in DDI water, likely due to the presence of constitutes that quench the autoradiolysis associated with decay of ^{14}C-TCE.

A propanotrophic culture that co-oxidized TCE was used as a positive control to evaluate the ^{14}C-TCE assay. ENV485 was used to assess the effect of storage conditions on the co-oxidation rate. It was determined that handling the culture in the same manner as the groundwater samples (i.e., 24 h at 4 °C to mimic shipping on ice, followed warming overnight to room temperature) caused a modest decrease in the first order rate coefficient. This suggests that the co-oxidation rates for the groundwater are conservative with respect to in situ conditions.

6.2.3 Quantification of Bacteria with Active Enzymes Associated with TCE Co-Metabolism

Quantitative polymerase chain reaction provides evidence for the presence of cometabolism genes in groundwater samples, while EAP provided lines of evidence that there are active cometabolic enzymes in a groundwater sample. Groundwater from five sites across the U.S. were analyzed using *q*PCR and EAP, and surprisingly few of the samples showed the presence and activity of the cometabolic oxygenase enzymes probed for during the project. Four of the 19 wells analyzed using the PA and 3HPA EAP, showed activity considered to be statistically significant (>8x10^3 cells/ml). Cinn only showed positive results for two of the nineteen wells tested.

In general, *q*PCR results corresponded to the EAP results for the PHE and RMO primer sets, but not for the TOD and TOL primer sets. Gene targets for sMMO were only detected significant levels (>10^3 cells/ml) at three of the 19 wells tested.

6.2.4 Demonstrate Ability to Predict TCE Co-Oxidation Rates by Quantifying Number of Bacteria with Active Co-Oxidation Enzymes

In the eight wells where biological co-oxidation could be distinguished from radiolysis, the Cinn EAP assay and the PHE and RMO *q*PCR determination provided a useful prediction of the rate constant for TCE co-oxidation, although the prediction intervals in the regression are broad. However, in many of the wells where co-oxidation was not detected, the abundance of cells reacting with the Cinn EAP and the abundance of DNA that is amplified by the PHE and RMO primer was high. The EAP and *q*PCR data do not provide an unequivocal prediction of the rate constant for TCE co-oxidation.

Because the Cinn EAP marker or the PHE and RMO *q*PCR markers do not provide an unequivocal prediction of the rate constant, the Cinn EAP marker or the PHE and RMO *q*PCR markers can only be used to identify groundwater where the predicted rate constants are possible. To use co-oxidation of TCE as part of a MNA remedy, it will be necessary to validate the predictions from the Cinn EAP marker or the PHE and RMO *q*PCR markers by obtaining rate constants using the ^{13}C-TCE assay or by extracting rate constants from the long-term monitoring data and the geohydrological properties of the aquifer.

If the groundwater used to perform the ^{13}C-TCE assay has measurable concentrations of Fe(II), it would be good practice to supplement and validate the rate constant produced by the assay with a rate constant that is extracted from the long-term monitoring data and the geohydrological properties of the aquifer.

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7.0 COST ASSESSMENT

This section provides information on the costs for implementing the various technology elements described in this report at a given site. In addition, this section provides a discussion of the cost benefit of the technology.

7.1 COST MODEL

A simple cost model for the technology is presented so that a remediation professional can understand costing implications. The cost model reflects all cost elements required for implementing the technology at a real site. For each cost element, the cost data that was tracked during the demonstration and the associated cost as incurred during the demonstration are presented.

Each cost element includes the following information:

- A description to briefly explain the cost element and the need for it in the implementation of the technology.
- A description and, if appropriate, supporting analysis as to what data supports the listed cost estimate or range.

A description of how issues of scale are addressed is included by providing per-well costs.

7.2 COST DRIVERS

Anticipated cost drivers in selecting the technology for future implementation are discussed in this cost assessment. The only site-specific characteristic that would significantly impact cost/implementability is if only 2inch wells that have been compromised are available for a given site. Wells that are considered compromised include those wells that are insufficiently straight to allow insertion and lowering of the downhole magnetic susceptibility sonde. In addition, compromised wells could have joints that are not flush, and the sonde cannot move past the joint. In such cases the sonde cannot be used and a drilling rig will have to be mobilized if the interested party wants to collect magnetic susceptibility data.

7.3 COST ANALYSIS

This section provides estimates for the costs of the technology when implemented. The basic site description for which costs were developed includes a site contaminated with chlorinated ethylenes that contains 2- or 4-inch monitoring wells installed to 100 feet in unconsolidated sediment or fractured rock. In order for the technology elements described in this document to be the most useful, monitoring wells used for sample collection should be located as near as possible to the plume centerline, parallel to the direction of solute flow.

This section describes and quantifies the operational costs for the various components of the technologies being developed and refined under this demonstration. For the purposes of developing the cost model presented herein, the technology has been broken down into cost elements, which are discussed and quantified in the following sections.

7.3.1 Cost Element 1 – Downhole Magnetic Susceptibility Measurements

This cost element includes mobilization of a two-person field crew to the field for three days to collect continuous downhole magnetic susceptibility measurements. Mobilization includes making all arrangements for equipment rental and site access. It is assumed that the wells to be analyzed have already been selected. Included in the costs for magnetic susceptibility measurements using a magnetic susceptibility sonde are the continuous sampling of up to eight wells to a depth of 100 feet bgs over a period of three days. These costs include the rental of all necessary equipment as well as labor hours. Table 7.1 summarizes the costs for this cost element. These costs can be scaled up and down by taking the mobilization cost, which includes the costs for renting and obtaining the equipment, of $1,200 and adding it to the per-well cost of $800 times the number of wells to be sampled. Thus, sonding one well would cost roughly $2,000, two wells would cost $2,800, and so on.

These costs are considerably cheaper than mobilizing a drilling rig specifically for the collection of soil/sediment cores for the analysis of magnetic susceptibility. This is the primary advantage of using the in-well sonde. If a drilling rig is already on site, samples can be obtained from the proper location, and continuous coring is already occurring, then using soil borehole samples analyzed in a fixed-based laboratory is probably the more economical way to go. As shown in this report, both the downhole sonde and soil borehole data analyzed in a fixed-base laboratory give similar results, so the choice of which methodology to utilize will depend primarily upon where the site characterization effort for the site stands. Because rental costs are fixed and well sampling must be completed at a specific rate, economies of scale are not likely to be significant with downhole sonde measurements.

7.3.2 Cost Element 2 – Groundwater Sample Collection for ^{14}C-Labeled TCE Assay, EAPS, and *q*PCR Analyses

This cost element includes mobilization of a two-person field crew to the field for three days to collect eight (8) groundwater samples for the ^{14}C-Labeled TCE assay as well as EAPs and *q*PCR analyses. Mobilization includes making all arrangements for equipment rental and site access. It is assumed that the wells to be analyzed have already been selected. Included in the costs are purging of up to three (3) casing volumes for wells up to 100 feet deep and the collection of DO, ORP, pH, temperature, and specific conductance data using a flow-through cell during well purging. It is assumed that sampling of these eight (8), 100-foot-deep wells will take three (3) days. These costs include the rental of all necessary equipment as well as labor hours. Table 7.1 summarizes the costs for this cost element. These costs can be scaled up and down by taking the mobilization cost of $1,400, which includes the costs for renting and obtaining the equipment, and adding it to the per-well cost of $1,220 times the number of wells to be sampled. Thus, sampling one well would cost roughly $2,620, two wells would cost $3,840, and so on. This cost element is required for implementation of cost elements 3, 4, and 5.

7.3.3 Cost Element 3 – ^{14}C-TCE Assay

The cost estimate is based on custom-synthesized ^{14}C-TCE and associated supplies, including liquid scintillation cocktail (LSC), liquid scintillation vials, supplies for the GC (gases, septa, syringes), serum bottles, and reagents. The cost of the custom synthesized ^{14}C-TCE is based on

1 mCi ($11,000) and a total of 500 bottles. Assuming triplicate bottles per groundwater sample, the sample cost of $66. Other material and supply costs are estimated at ~$150 per sample, for a total supply cost of ~$216 per sample.

Staff labor to perform the assay was estimated at four hours per sample (from preparation through clean-up, plus data reduction). At $50/hr, the staff labor is estimated at $200 per sample. Time for supervisory labor is estimated at 10% of the staff and $150/hr, or $60 per sample. This brings the total cost to $476 per sample.

As the assay matures, there will no doubt be opportunities to bring the costs down. This has certainly been the experience with application of molecular tools to groundwater contamination. Regardless, the pay-off from using the ^{14}C-assay may be considerable. An assay based on ^{14}C product accumulation from ^{14}C-TCE affords an opportunity to determine realistic decay rates that can be used in groundwater models to predict if MNA will be successful. Absent this type of information, there is considerable uncertainty in what constitutes an appropriate rate to use in modeling.

7.3.4 Cost Element 4 – EAPs

EAP and *q*PCR will be applied to groundwater samples taken from monitoring wells. This cost element includes sample bottles and material for sample processing for shipment to lab, EAP probes, filters, microscope slides, other reagents required for sample preparation. This cost analysis assumes that equipment such as vacuum pumps or house vacuum supply, fluorimeter (coumarin) and a microscope with epifluorescence capability is available for use. Total supplies costs for eight samples will be approximately $200.

Staff labor is estimated at five hours per probe per sample. For eight groundwater samples and triplicate analysis per sample, a total of 20 hours will be required for the analysis, which includes sample filtration and manual enumeration using epifluorescence microscopy. Blanks, positive controls and matrix spikes are included in per unit cost for the analyses, so these analyses will require an additional 20 hours per sample. The time for the project Principal Investigator is approximately 10% of the staff time, or 4 hours. Staff time for data reduction is estimated to require 10% of the analysis time, or 4 hours. The time for the project Principal is estimated to be 5% of the analysis time, or 2 hours. Table 7.1 summarizes the costs for this cost element. These costs are for the demonstration and include PNNL cost schedule and burden rates. For analysis of eight samples with four EAP (3HPA, PA, Cinn and coumarin), and counterstaining with 4′,6-Diamidino-2-phenylindole dihydrochloride (DAPI) by a commercial laboratory or university core facility is estimated to be approximately $400 per EAP per sample. Eight samples could be analyzed for $13,000 which includes the supplies estimate from above. Adding the costs for analyzing the data (data reduction and reporting), the per-sample cost for this analysis is about $1,900. Table 7.1 summarizes the costs for this cost element. These costs are for this demonstration. It is anticipated that unit costs will decrease significantly if this process is commercialized.

Because the Cinn EAP marker does not provide an unequivocal prediction of the rate constant, the Cinn EAP marker can only be used to identify groundwater where the predicted rate constants are possible.

7.3.5 Cost Element 5 – *q*PCR Analyses

This cost element includes sample bottles and material for sample processing for shipment to lab, DNA extraction kits, filters, oligonucleotide primers, DNA polymerase core kits, 96-well PCR plates and other reagents required for sample preparation and preservation.

Due to the nature of the analyses, blanks, standards, and positive controls can be processed in parallel in the same PCR plate. Blanks, and positive controls are included in per unit cost for the analyses and do not require additional time. Staff time for data reduction and reporting is estimated to require 8 hours. The time for the project Principal Investigator is estimated to be 3 hours.

Table 7.1 summarizes the costs for this cost element, not including sample collection costs. These costs include CENSUS-DNA data and are based on a cost estimate from Microbial Insights in Knoxville, TN. CENSUS-DNA data included in the costs presented in Table 7.1 include costs for:

- Toluene Monooxygenase--RMO
- Toluene Monooxygenase 2--RDEG
- Phenol Hydroxylase--PHE
- Toluene Dioxygenase--TOD
- Xylene Monooxygenase--TOL
- Soluble Methane Monooxygenase--SMMO

The per-sample cost for these analyses is roughly $835 per sample, including data analysis and reporting costs. The benefit of the *q*PCR analyses is that they allow the user to determine if aerobic cometabolism is possible.

Because the PHE and RMO qPCR markers do not provide an unequivocal prediction of the rate constant, the PHE and RMO qPCR markers can only be used to identify groundwater where the predicted rate constants are possible.

Table 7.1. Cost Model for Eight Samples Each for Magnetic Susceptibility, 14C-Labelled TCE Assay, EAPs and qPCR

Cost Element	Administration/ Secretarial $60 (per hour)	Drafting $90 (per hour)	Staff Environmental Professional $100 (per hour)	Senior Environmental Professional $135 (per hour)	Principal $200 (per hour)	Other Direct Cost[a/]	Number of Samples or Days of Rental (total)		Other Direct Costs Subtotal	Subtotal - Labor Plus ODCs
1 - Downhole Magnetic Susceptibility Measurements[b/]										
Mobilization			10		1					$ 1,200
Two Person Field Crew for Two (2) Days			32							$ 3,200
Sonde, Tripod, Winch, Data Logger (including shipping time)			2		1	$400	3	daily	$1,200	$ 1,600
Data Reduction and Reporting			8		4					$ 1,600
									TASK SUBTOTAL	**$ 7,600**
2 - Groundwater Sample Collection for ^{14}C-Labeled Assay, EAPs, and qPCR Analyses[c/]										
Mobilization			10		2					$ 1,400
Two Person Field Crew for Three Days			60		2					$ 6,400
Grundfos Pump, Generator, Meter for DO, pH, Temp, spec. cond., ORP, and Fe(II), Health and Safety Equipment, etc.			2			$500	3	daily	$1,500	$ 1,700
Data Reduction and Reporting	4		8		3					$ 1,640
									TASK SUBTOTAL	**$ 11,140**
3 - ^{14}C-Labeled TCE Assay[d/]										
^{14}C-TCE + associated supplies; triplicates for each sample from a monitoring well. Includes all labor costs.						$476	8	each	$3,808	$ 3,808
Data Reduction and Reporting	1		8		3			each		$ 1,460
									TASK SUBTOTAL	**$ 5,268**
4 - Enzyme Activity Probes[d/]										
Four Probes Per Sample, Eight (8) samples					4	$1,625	8	each	$13,000	$ 13,800
Data Reduction and Reporting	1		8		3			each		$ 1,460
									TASK SUBTOTAL	**$ 15,260**
5 - *p*PCR Analyses[d/]										
CENSUS-DNA (Toluene Monooxygenase--RMO)						$275	8	each	$2,200	$ 2,200
CENSUS-DNA (Toluene Monooxygenase 2--RDEG)						$75	8	each	$600	$ 600
CENSUS-DNA (Phenol Hydroxylase--PHE)						$75	8	each	$600	$ 600
CENSUS-DNA (Toluene Dioxygenase--TOD)						$75	8	each	$600	$ 600
CENSUS-DNA (Xylene Monooxygenase--TOL)						$75	8	each	$600	$ 600
CENSUS-DNA (Soluble Methane Monooxygenase--SMMO)						$75	8	each	$600	$ 600
Data Reduction and Reporting	1		8		3			each		$ 1,460
									TASK SUBTOTAL	**$ 6,660**
Totals	7		**156**		**26**				**Total Labor Costs**	**$ 21,220**
									Total ODCs	**$ 24,708**
									Total Estimated Cost =	**$ 45,928**

Notes and Assumptions

a/ Other direct cost in the form of laboratory analyses, field analyses, or equipment rental

b/ Assumes sonding of 8 boreholes to depths of 100 feet below ground surface.

c/ Assumes purging 3 casing volumes from 8 groundwater monitoring wells that are completed to a depth of 100 feet below ground surface and collecting groundwater samples for submission to the analytical laboratory.

d/ Does not include the cost of obtaining the sample, the costs for which are included under Item 2, Groundwater Sample Collection.

8.0 IMPLEMENTATION ISSUES

This section provides information that will aid in the future implementation of the technology. A brief description and references for other documents such as guidance or protocols are provided. The technology elements described in this document were developed or refined because of shortcomings identified in ESTCP (2015). Specifically, the lack of a way to quantify the degradation of the chlorinated ethylenes by magnetite after intrusive site characterization activities (i.e., drilling) had been completed, and the lack of a method to conclusively show and quantify aerobic cometabolism of TCE. The technologies presented in this report represent an improvement over that presented in ESTCP (2015).

Lessons learned during the demonstration are as follows:

- The only regulations that apply to the use of the technologies presented in this report are the permits required to use of the ^{14}C assay. The analytical laboratory must obtain certification in order to handle ^{14}C.
- The magnetic susceptibility sonde provides a readily-accessible and accurate alternative to intrusive soil borehole data collection for magnetic susceptibility.
- The magnetic susceptibility sonde cannot be used in stainless steel wells. Wells larger than 4 inches in diameter may be problematic for collecting accurate magnetic susceptibility data using the sonde identified in this report because of the size of the borehole required for such wells. However, larger sondes, with a larger radius of influence, are available.
- Metallic tools or other metal objects dropped into boreholes in which monitoring wells are subsequently installed will interfere with the magnetic susceptibility sonde. Steel centralizers used to keep a casing or screen in the center of the borehole will have the same effect. The spike in magnetic susceptibility is very strong, and it is easy to identify the depth intervals that are affected by extraneous ferrous metal in the well. The reading for magnetic susceptibility in the depth intervals that are affected can/should be deleted from the record before the data are father analyzed.
- As mentioned above, ^{14}C assays can only be performed in laboratories that are permitted to use radioactive material. Furthermore, the cost for ^{14}C-labeled TCE is considerable (~$11,000 per mCi), mainly because it is no longer available as a stock compound and must therefore be custom synthesized. If the assay is adopted for more frequent use, suppliers may opt to once again provide ^{14}C-labeled TCE as a stock item, which will decrease the cost.
- The ^{14}C assay is not yet commercialized. It is hypothesized that the successful demonstration of the protocol presented in this report will provide considerable motivation for private companies to offer the service. An analogous situation was the use of compound specific isotope analyses (CSIA). At one time, use of this technology for groundwater samples was limited to a select few academic laboratories. As the value of the approach became apparent, commercial laboratories stepped in to meet the growing demand. We anticipate that a similar outcome will develop for the ^{14}C assay proposed in this study.

- EAP analytical services are currently only available through PNNL.
- *q*PCR can be affected by biases associated with DNA extraction, as well as issues associated with efficiency of DNA amplification.

Overall, implementation issues are negligible, and the technologies presented herein should allow the decision framework (and BioPIC) presented in ESTCP (2015) to be updated so that additional degradation pathways can be readily elucidated and quantified.

9.0 REFERENCES

ESTCP, 2016, Demonstration Plan: Providing Additional Support for Monitored Natural Attenuation by Including Quantitative Lines of Evidence for Abiotic Degradation and Co-Metabolic Oxidation of Chlorinated Ethylenes: ESTCP Project ER-201584.

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APPENDIX A POINTS OF CONTACT

Point of Contact Name	Organization Name	Phone Email	Role in Project
Todd Wiedemeier (Deceased)	T.H. Wiedemeier & Associates, Inc.	(303) 670-7999 todd@thwa.com	Principal Investigator
Dr. John T. Wilson	Scissortail Environmental, LLC	(580) 421-3551 john@scissortailenv.com	Senior technical advisor to the team on MNA and abiotic processes, participation in field work, and report development.
Dr. David L. Freedman	Clemson University	864-656-5566 dfreedm@clemson.edu	Senior technical advisor on aerobic microbial aspects. Lead on ^{14}C-labeled TCE assay for degradation rates.
Dr. Brady Lee	Pacific Northwest National Laboratory (PNNL) (Federal Partner)	509-375-4593 brady.lee@pnnl.gov	Senior technical advisor for EAPs and *q*PCR assays.

ESTCP Office
4800 Mark Center Drive
Suite 17D08
Alexandria, VA 22350-3605
(571) 372-6565 (Phone)
E-mail: estcp@estcp.org
www.serdp-estcp.org